Miltenyi Biotec

CliniMACS® Plus Applications

User Manual

The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485. In the EU, the CliniMACS System components are available as CE-marked medical device for their respective intended use, unless otherwise stated. In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set TS and CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Instrument (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational device Exemption (IDE) or FDA approval. In Australia, the following components of the CliniMACS Plus System are included in the Australian Register of Therapeutic Goods (ARTG) and are therefore approved for supply: CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set, CliniMACS Tubing Set LS, CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer. Only those products which are included in the ARTG may be used in Australia.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use.

Copyright © 2023 Miltenyi Biotec and/or its affiliates. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system, transmitted, published, or distributed in any form or by any means, electronically, mechanically, by photocopying, microfilming, recording, or otherwise, without the prior written consent of Miltenyi Biotec; however, notwithstanding the foregoing, the owners of the CliniMACS Plus System may make copies solely for purposes of training personnel in the use and servicing of the unit within their business or organization.

CliniMACS, MACS, MACSmix, the Miltenyi Biotec logo, and MiniMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only.

CliniMACS® Plus Applications

User Manual

Software version 2.4x

Issued: 2023-02
210-003-187/03

MD CE 0123

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

Miltenyi Biotec Technical Support
+49 2204 8306-3803
technicalsupport@miltenyi.com

www.miltenyibiotec.com

Essential information

This user manual provides information for the use of the CliniMACS Plus Applications. Instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Instrument are provided in the CliniMACS Plus Instrument User Manual.

⚠ WARNING

The operation of the CliniMACS Plus System must be performed by trained operators only. Before putting the sytem into operation, carefully read and understand the safety information, warnings, precautions, and instructions for proper operation of the CliniMACS Plus Instrument provided in the CliniMACS Plus System instructions for use (including, without limitation, the safety information in the CliniMACS Plus Instrument User Manual and chapter 3 "Warnings and precautions" on page 27 in this user manual) and in any safety-related recommendations issued by Miltenyi Biotec. Pay special attention to all warnings shown on the instrument or provided with consumables, accessories, additional materials and equipment. The operator must adhere to all safety information, warnings, precautions, and instructions at all times during the operation of the instrument, confirming that all safety information, warnings, precautions, and instructions are observed. Failure to follow the safety information, warnings, precautions, and instructions contained in the instructions for use could result in instrument malfunction, property damage, loss of target cells, personal injury, and/or death.

Table of contents

1 Introduction

1.1 General information

The CliniMACS Plus System offers a set of tools making high-quality standard cell separations available for therapeutic applications.

The CliniMACS Plus System is based on the magnetic cell separation technology (MACS® Technology) developed by Miltenyi Biotec B.V. & Co. KG. Miltenyi Biotec has made these products available for clinical applications meeting the requirements of European Regulatory Standards.

The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients.

The CliniMACS Reagents in combination with the CliniMACS Plus System are intended to separate human cells.

Miltenyi Biotec as the manufacturer of the CliniMACS Plus System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit.

For the manufacturing and use of target cells in humans the national legislation and regulations – e.g., for the EU the Directive 2004/23/EC (human tissues and cells) or the Directive 2002/98/ EC (human blood and blood components) – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS Plus System.

LIMITED WARRANTY
Should the CliniMACS Plus System be used in a manner not explicitly described in this manual, all warranties will be null and void.

IMPORTANT
Before using the CliniMACS Plus System or any components outside the European Economic Area, the regulatory approval of the CliniMACS Plus System or any CliniMACS Component in the country must be confirmed.

1.2 Labeling of cells with CliniMACS Reagents

MACS Magnetic Cell Sorting is a well proven powerful tool for the separation of many cell types, in research laboratories as well as in clinical applications. Cell mixtures are separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, e.g., human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

Using the CliniMACS Plus System cells can be magnetically labeled in three different ways:

- Directly in a one-way labeling procedure by antigen-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see Figure 1.1).
- Indirectly in a two-step labeling procedure called Flexible Labeling System. In a first step cells are labeled with antigen-specific antibodies conjugated to biotin. In a second step these antibodies are labeled with biotin-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see Figure 1.2).
- Indirectly in a two-step labeling procedure called CliniMACS Cytokine Capture System (IFN-gamma). First of all, cells are *in vitro* restimulated to secrete the cytokine (e.g., IFN-gamma). In a first labeling step the re-stimulated cells are labeled by a hybrid-molecule consisting of a leucocyte-specific antibody and a cytokine-specific antibody, called the CliniMACS IFN-gamma Catchmatrix Reagent. The cells are then incubated to allow secretion of the cytokine, which is "captured" by the catchmatrix on the cell surface. Sub-sequently, in a second labeling step the cytokine secreting cells are labeled with cytokine-specific antibodies conjugated to superparamagnetic iron-dextran beads (CliniMACS IFN-gamma Enrichment Reagent), (see Figure 1.3).

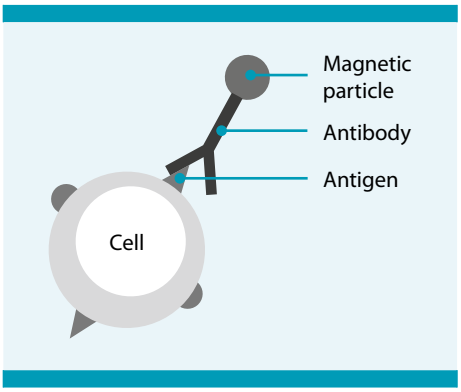


Figure 1.1: Magnetic labeling of cells

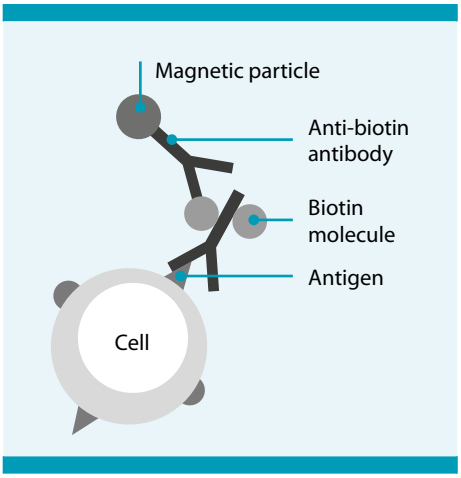


Figure 1.2: Flexible Labeling System

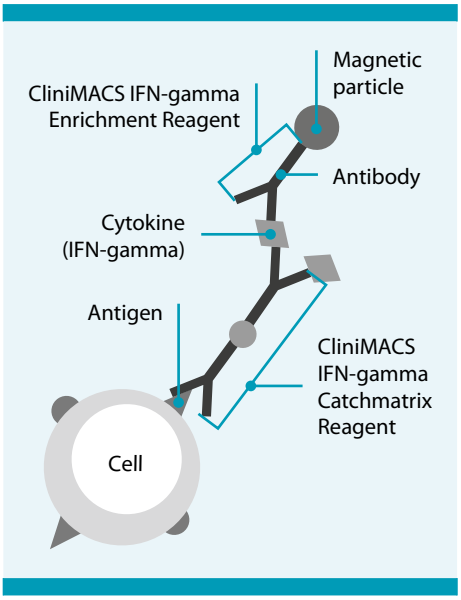


Figure 1.3: CliniMACS Cytokine Capture System (IFN-gamma)

1.3 High-gradient magnetic cell separation

The magnetically labeled cell suspension is loaded onto the CliniMACS Plus Instrument prepared with a tubing set. This high-gradient magnetic cell separation unit consists of a specifically developed, powerful permanent magnet and a separation column with a ferromagnetic matrix.

The high-gradient field allows the generation of strong magnetic forces and a rapid demagnetization. When small ferromagnetic structures, such as the column matrix, are placed within the magnetic field they disrupt the homogeneity of the field. This results in the generation of high magnetic gradients. In their immediate surrounding the ferromagnetic structures generate magnetic forces 10,000-fold stronger than in conventional geometries. The high-gradient field attracts labeled cells to the matrix and effectively retains them. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

1.4 Separation strategies

The CliniMACS Plus System provides the user with a variety of separation programs. The separation programs can generally be divided into enrichment strategies (CD34 SELECTION 1 and 2, ENRICHMENT 1.1, and ENRICHMENT 3.2) and depletion strategies (DEPLETION 2.1 and DEPLETION 3.1).

Enrichment of magnetically labeled cells

When choosing an enrichment strategy, the magnetically labeled cells (primary labeled with a CliniMACS Reagent) are retained in the separation column and the non-labeled cells pass through. The labeled cells (target cells) are collected in the Cell Collection Bag and the non-labeled cells (non-target cells) in the Negative Fraction Bag (see Figure 1.4).

Depletion of magnetically labeled cells

The MACS Technology is also very efficient for depleting specific cell populations. Unwanted cells are specifically labeled with super-paramagnetic particles and separated from target cells upon passage through the high-gradient magnetic column. In contrast to the enrichment strategy, the non-labeled cells (target cells) are collected in the Cell Collection Bag and the labeled cells (non-target cells) are collected in the Negative Fraction Bag (see Figure 1.5) or in the Non-Target Cell Bag (see Figure 1.6 and Figure 1.7) respectively.

1.4.1 Enrichment strategy

Enrichment of cells using the CliniMACS Tubing Set or CliniMACS Tubing Set LS

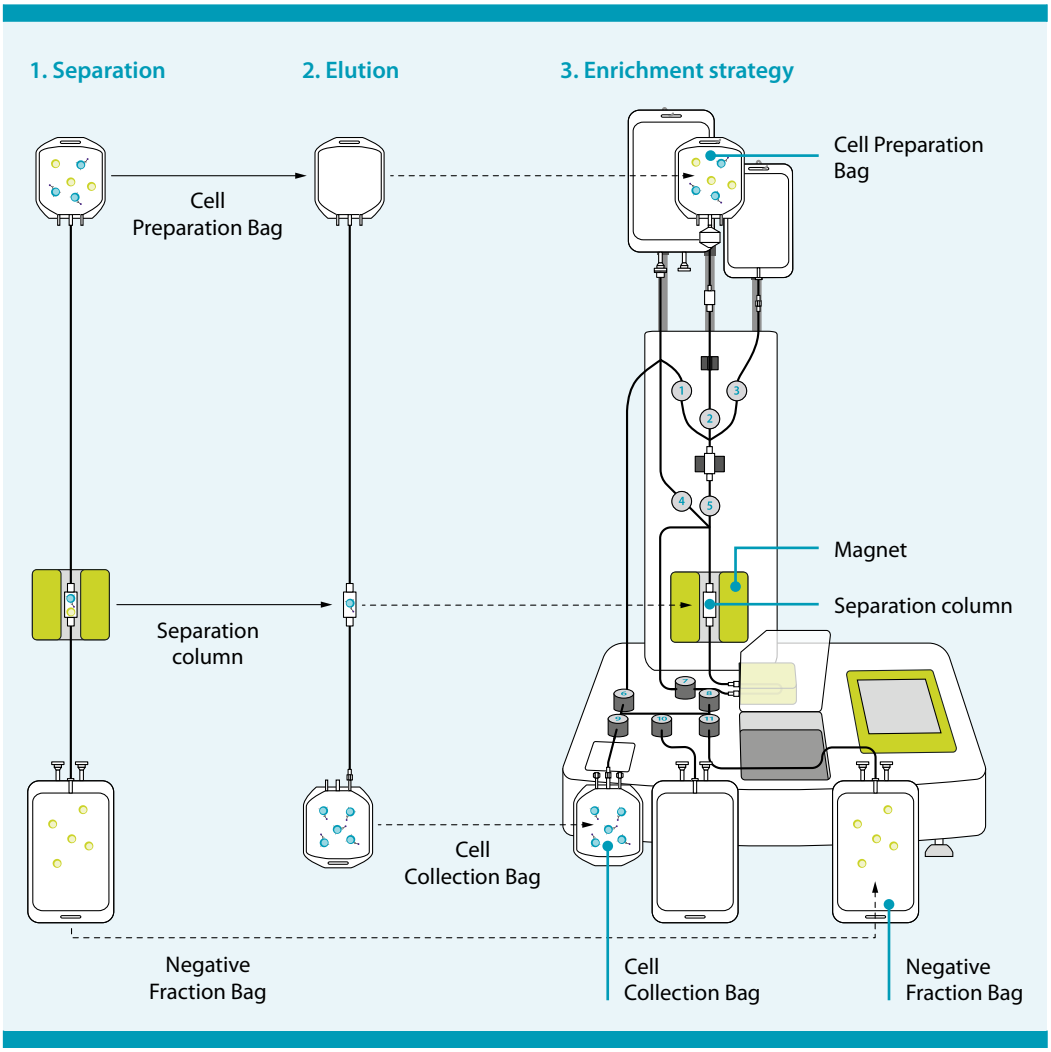


Figure 1.4: Strategy of enrichment programs using the CliniMACS Tubing Set or CliniMACS Tubing Set LS

1. The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag.
2. The magnet is in the "OFF"-position. The magnetically labeled cells (target cells) are released from the separation column and collected in the Cell Collection Bag.
3. The enrichment program retains the magnetically labeled cells in the separation column, the non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag. When the magnet is moved into the "OFF"-position, the magnetically labeled cells (target cells) are released from the column and collected in the Cell Collection Bag. **Note:** The target cell fraction is always collected in the Cell Collection Bag.

1.4.2 Depletion strategy

Depletion of cells using the CliniMACS Tubing Set LS: DEPLETION 2.1

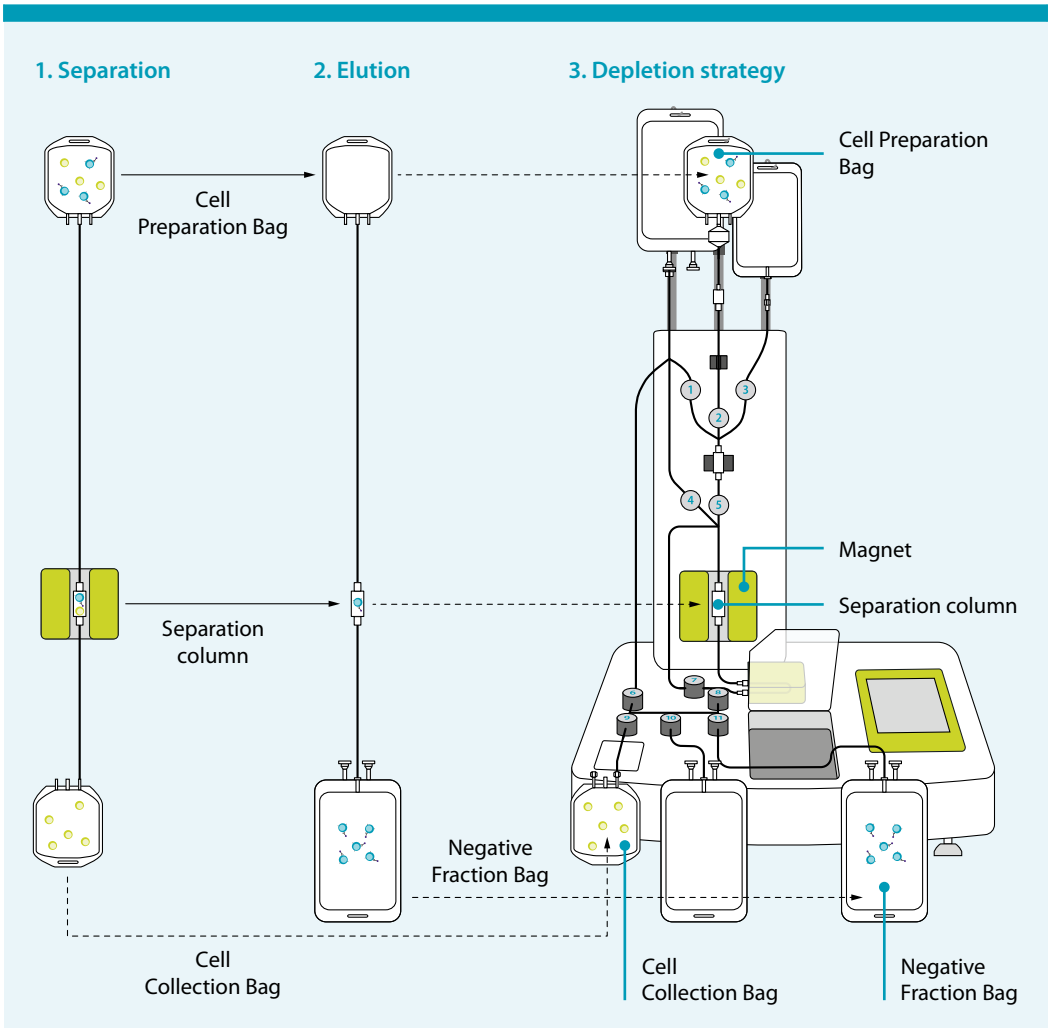


Figure 1.5: Strategy of the depletion program DEPLETION 2.1 using the CliniMACS Tubing Set LS

1. The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.
2. The magnet is in the "OFF"-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Negative Fraction Bag.
3. The depletion program (DEPLETION 2.1) retains the magnetically labeled cells in the separation column, the non-labeled cells (target cells) flow through and are collected in the Cell Collection Bag. When the magnet is moved into the "OFF"-position, the magnetically labeled cells (non-target cells) are released from the column and collected in the Negative Fraction Bag. **Note:** The target cell fraction is always collected in the Cell Collection Bag.

Depletion of cells using the CliniMACS Depletion Tubing Set: DEPLETION 3.1 (Part 1: Staged Cell Loading [Bulk Loading Stage])

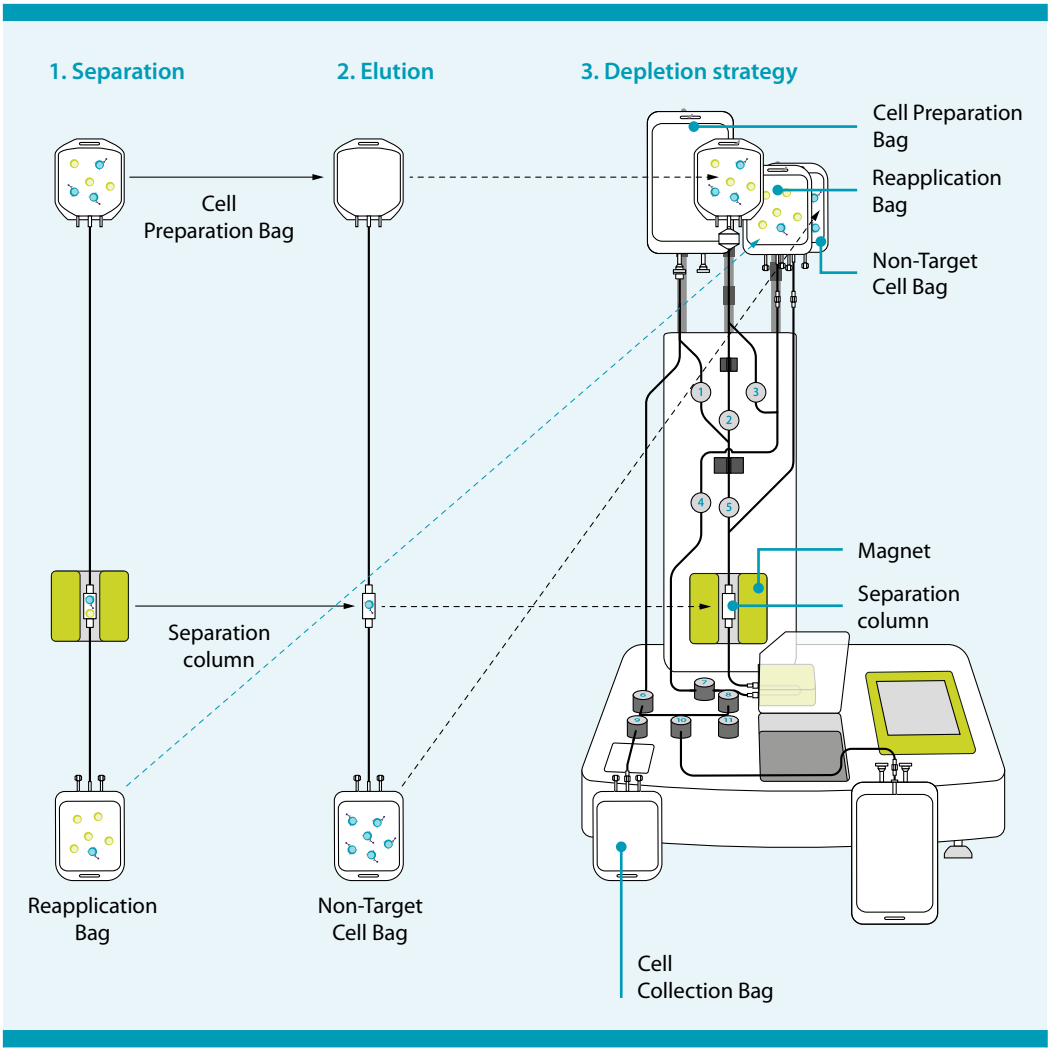


Figure 1.6: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 1)

1. Staged Cell Loading (Bulk Loading Stage): The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through and are collected in the Reapplication Bag.
2. The magnet is in the "OFF"-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Non-Target Cell Bag.
3. The depletion program (DEPLETION 3.1) retains the magnetically labeled cells in the separation column, the non-labeled cells (target cells) flow through the column and are collected in the Reapplication Bag in order to be reloaded onto the separation column in a second sensitive depletion (see Figure 1.7). When the magnet is moved into the "OFF"-position, the magnetically labeled cells (non-target cells) are released from the column and collected in the Non-Target Cell Bag.

Depletion of cells using the CliniMACS Depletion Tubing Set: DEPLETION 3.1 (Part 2: Sensitive Sample Loading [Sensitive Loading Stage])

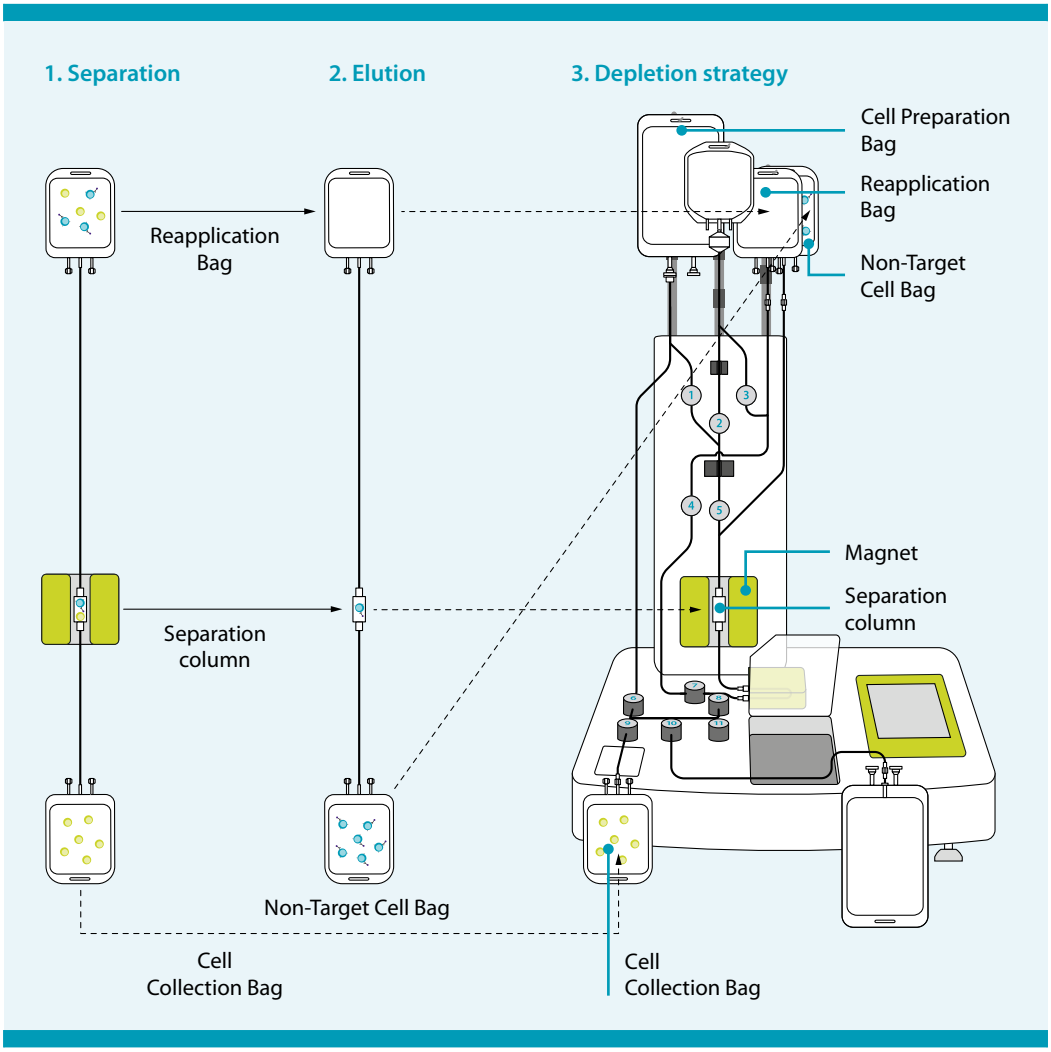


Figure 1.7: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 2)

4. Sensitive Sample Loading (Sensitive Loading Stage): The magnet is in the "ON"-position. The cells from the Reapplication Bag are reloaded on the separation column. The few remaining magnetically labeled cells are held in the separation column, while the non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.

5. The magnet is in the "OFF"-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Non-Target Cell Bag.

6. At the end of the separation the labeled cells (non-target cells) are in the Non-Target Cell Bag and the non-labeled cells (target cells) are in the Cell Collection Bag. **Note:** The target cell fraction is always collected in the Cell Collection Bag.

2 Glossary

2.1 Graphical depiction

The following chart depicts the panels used in this user manual to inform the user about potential risks if the outlined warnings and precautions are not followed. The hazard level classifies the hazard, as described below. The level, type, and source of the hazard, as well as potential consequences, prohibitions, and measures are indicated as follows. Icons on the left side specify the risk.

⚠ WARNING

Indicates a hazardous situation that, if not avoided, could result in death or serious injury

⚠ CAUTION

Indicates a hazardous situation that, if not avoided, could result in minor or moderate injury

NOTICE

Indicates information considered important, but not hazard-related (e.g. messages relating to property damage)

IMPORTANT

Advises the user of important practices or information not related to personal injury nor property damage

2.2 Glossary of symbols

An overview of symbols used for the CliniMACS Plus System is provided in the CliniMACS Plus Instrument User Manual. The glossary of symbols depicts the symbols used in the user manuals and labeling of the CliniMACS Products.

2.3 Glossary of terms

Apheresis	The method of collecting blood in which whole blood is withdrawn, a desired component selected and retained, and the remainder of the blood returned to the donor
Anti-biotin antibody	This antibody recognizes cells which have previously been labeled with an appropriate biotinylated antibody or ligand.
Bag compartment	Compartment of the CliniMACS Plus Instrument in which the Negative Fraction Bag and Buffer Waste Bag are placed
Bag hanger	Support on the CliniMACS Plus Instrument to mount the Cell Preparation Bag, Non-Target Cell Bag, Priming Waste Bag, Reapplication Bag, and buffer bag
BDCA	Blood Dendritic Cell Antigen
Buffer Waste Bag	Waste bag to collect buffer during cell separation using the CliniMACS Plus Instrument
CD1c (BDCA-1) antigen	CD1c is part of a CD1 gene cluster that is believed to be related to the MHC. The CD1 genes are clustered within a 170 000 bp region of human chromosome 1q23.1. The proteins encoded therein (CD1a-e) are expressed as heterodimers composed of CD1 heavy chain non-covalently paired with ß2-microglobulin. CD1c is involved in antigen presentation of microbial glycolipids or lipopeptide antigens to T lymphocytes. In human peripheral blood, CD1c (BDCA-1) is expressed on myeloid dendritic cells type-1 (MDC1) and on a subset of B lymphocytes. They can be found in lymph node, mantle zones and germinal centers, in marginal zone of spleen and in fetal and adult human peripheral blood.
CD3 antigen	The CD3 antigen is present on mature human T cells, thymocytes and a subset of NK cells. It is associated with the T cell receptor (TCR) and is responsible for the signal transduction of the TCR. The CD3 antigen is a complex of five invariable chains: γ, δ, ε, ζ and η. The CD3 antibody recognizes all T cells, i.e. it reacts with 70-80% of human peripheral blood lymphocytes and with 65-85% of thymocytes. The epitope recognized by the antibody is located on the ε-chain of the CD3 complex.
CD4 antigen	The CD4 antigen is expressed on most thymocytes and approximately two-thirds of peripheral blood T cells; it is also expressed on monocytes and macrophages. CD4 is an accessory molecule in the recognition of foreign antigens in association with MHC class II. Additionally, CD4 is a receptor for HIV-1.

CD8 antigen	The CD8 antigen is a disulfide-linked dimer, which exists either as a CD8 α homodimer or as a CD8 α/β heterodimer. CD8 is strongly expressed on CD8 positive T cells and thymocytes and on a subset of NK cells and CD8 positive γ/δ T cells. CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition.
CD14 antigen	The CD14 antigen is a 53-55 kD cell membrane glycoprotein expressed on monocytes. The CD14 antigen is known as a receptor for complex of LPS and LPS binding protein (LBP).
CD19 antigen	The CD19 antigen is a critical signal transduction molecule that regulates B lymphocyte development, activation, and differentiation. As a B cell lineage marker, CD19 is expressed from the early pro-B cell stage to the B cell lymphoblast stage but the expression is downregulated upon B cell maturation to plasma cells. The CD19 antigen is further expressed on most malignant B cells and a subset of follicular dendritic cells.
CD25 antigen	The CD25 antigen, the low affinity interleukin-2 receptor alpha chain (IL-2Rα), is expressed on activated T and B cells, NK cells and monocytes, as well as on regulatory CD4 positive T cells. The CD25 antigen shows three epitope regions A, B and C. The antibody of the CliniMACS CD25 Reagent recognizes epitope A of the CD25 molecule.
CD34 antigen	The CD34 antigen is a highly glycosylated 115 kDa type 1 integral membrane protein of unknown function which is expressed on 1% to 4% of normal bone marrow cells and less than 0.2% of normal peripheral blood leukocytes, on subsets of bone marrow stromal cells, and on small vessel endothelium of various tissues.
CD45RA antigen	The CD45RA antigen is expressed on naive CD4 and CD8 positive T cells, as well as on subsets of B cells, NK cells, and monocytes. It is an isoform of the common leukocyte antigen CD45, a transmembrane protein tyrosine phosphatase.
CD56 antigen	The CD56 antigen is a member of the NCAM-family (Neural Cell Adhesion Molecule-family) which is expressed within the hematopoietic system on NK, NKT cells.
CD304 (BDCA-4) antigen	The CD304 (BDCA-4) antigen is a 130-140 kDa cell surface glycoprotein expressed in fresh blood and bone marrow exclusively on plasmacytoid dendritic cells.
Cell Collection Bag	Bag in which the purified target cells are accumulated after separation
Cell Preparation Bag	Bag into which cell product is transferred and in which magnetic labeling and washing of cells are performed

Cellular starting product	Cell-containing product used as starting material for the CliniMACS Plus Separation process, e.g., leukapheresis harvest, PBMCs, or bone marrow
CliniMACS Anti-Biotin Reagent	Reagent for magnetic labeling of cells primarily labeled with biotinylated antibodies or ligands.
CliniMACS CD1c Biotin (BDCA-1)	Biotinylated antibody for labeling of human cells expressing the CD1c (BDCA-1) antigen prior to the magnetic labeling with the CliniMACS Anti-Biotin Reagent.
CliniMACS CD3 Reagent	Reagent for magnetic labeling of cells expressing the CD3 antigen
CliniMACS CD4 Reagent	Reagent for magnetic labeling of cells expressing the CD4 antigen
CliniMACS CD8 Reagent	Reagent for magnetic labeling of cells expressing the CD8 antigen
CliniMACS CD14 Reagent	Reagent for magnetic labeling of cells expressing the CD14 antigen
CliniMACS CD19 Reagent	Reagent for magnetic labeling of cells expressing the CD19 antigen
CliniMACS CD25 Reagent	Reagent for magnetic labeling of cells expressing the CD25 antigen
CliniMACS CD34 Reagent	Reagent for magnetic labeling of cells expressing the CD34 antigen
CliniMACS CD45RA Reagent	Reagent for magnetic labeling of cells expressing the CD45RA antigen
CliniMACS CD56 Reagent	Reagent for magnetic labeling of cells expressing the CD56 antigen
CliniMACS CD304 (BDCA-4) Reagent	Reagent for magnetic labeling of cells expressing the CD304 (BDCA-4) antigen
CliniMACS Cytokine Capture System (IFN-gamma)	Combination of reagents, consisting of the CliniMACS IFN-gamma Catchmatrix Reagent and the CliniMACS IFN-gamma Enrichment Reagent. With the CliniMACS Cytokine Capture System (IFN-gamma), IFN-gamma secreting cells can be enriched after *in vitro* restimulation.

CliniMACS Depletion Tubing Set	Set of tubing, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place, especially designed for the specific depletion needs
CliniMACS IFN-gamma of Catchmatrix Reagent	Component of the CliniMACS Cytokine Capture System (IFN-gamma) for surface labeling leukocytes with IFN-gamma specific antibodies
CliniMACS IFN-gamma Enrichment Reagent	Component of the CliniMACS Cytokine Capture System (IFN-gamma) for magnetic labeling of IFN-gamma bound to the CliniMACS IFN-gamma Catchmatrix Reagent on the IFN-gamma secreting cells
CliniMACS PBS/EDTA Buffer	Buffer used for cell preparation and cell separation with the CliniMACS Plus System: PBS (phosphate buffered saline), supplemented with 1 mM EDTA, pH 7.2. Before use, CliniMACS PBS/EDTA Buffer must be supplemented with pharmaceutical grade HSA to a final concentration of 0.5% (weight/volume, i.e., 5 g HSA per liter buffer).
CliniMACS Plus Instrument	Magnetic cell separation instrument based on the MACS Technology
CliniMACS TCRα/β-Biotin	Biotinylated antibody for labeling of human cells expressing the TCRα/β antigen prior to the magnetic labeling with the CliniMACS Anti-Biotin Reagent
CliniMACS Tubing Set, CliniMACS Tubing Set LS	Set of tubing, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place
EDTA	Ethylene-diamine-tetra-acetic acid
×g	Multiples of the earth's gravitational acceleration
Heat sealer	Heating device used to sterile seal PVC tubing
Hematopoietic progenitor cells	Progenitor cells of lymphoid, myeloid, and erythroid lineages
HSA	Human serum albumin
Human serum albumin	Pharmaceutical grade HSA approved in the country of the user is necessary as a buffer supplement when used with the CliniMACS Plus System.
IgG	Immunoglobulin G

In-bag procedure	Sterile processing and transferring the cells in the closed environment of transfer bags
Labeling	Reaction of cells with magnetic labeling reagent, e.g., CliniMACS CD34 Reagent to CD34 positive cells
Leukapheresis	Apheresis collecting leukocytes
Liquid sensor	Component of the CliniMACS Plus Instrument that detects liquid in the tubing
Luer connector	Screw coupling, part of the tubing set
Luer/Spike Interconnector	Transfer set with piercing pin (spike) and syringe adapter (female luer) for plasma or fluids
Magnetic antibody	A super-paramagnetically labeled antibody
MDC	Myeloid dendritic cells
Monoclonal antibodies	A single type of antibody that is directed against a specific epitope (antigen, antigenic determinant) and is produced by a single clone of B cells or a single hybridoma cell line, which is formed by the fusion of a lymphocyte cell with a myeloma cell. Some myeloma cells synthesize single antibodies naturally.
Negative Fraction Bag	Bag of the CliniMACS Tubing Set and CliniMACS Tubing Set LS containing the non-target cell fraction
Non-Target Cell Bag	Bag of the CliniMACS Depletion Tubing Set containing the non-target cell fraction
Orbital rotator	Device used to mix cell product during the reaction with CliniMACS Reagents
PBMC	Peripheral Blood Mononuclear Cell
Peristaltic pump	Tubing pump used in the CliniMACS Plus Instrument to control the flow rate of fluid in the tubing set
Plasma extractor	Device used to extract liquid from the Cell Preparation Bag after cell washing
Plasma transfer set	Transfer set with two piercing pins (spikes) for plasma or fluids
Plasma Waste Bag	Waste bag to collect excess plasma prior to the labeling procedure

Pre-column	First column in the CliniMACS Tubing Set and the CliniMACS Tubing Set LS, serves as filter to trap cells having non-specific interactions with the column matrix
Pre-column holder	Support mounted on the CliniMACS Plus Instrument that holds the pre-column in place
Pre-system filter	40 µm filter device between Cell Preparation Bag and pre-column used to trap clumps and cell debris
Priming	Step prior to cell separation in which buffer is flushed through the tubing set
Priming Waste Bag	Bag in which buffer from priming step is collected
Pump safety switch	Sensor that prevents pump operation when the pump door is open
Reapplication Bag	Bag of the CliniMACS Depletion Tubing Set in which the non-labeled cells are collected temporarily during the separation. The non-labeled cells from the Reapplication Bag are applicated onto the separation column twice, to ensure high purity of the target cells.
Retaining ring	Part of a tubing set that enables the pump tubing to remain in its proper location
rpm	Revolutions per minute
Sampling site coupler	Injection port, e.g., for removal of samples or addition of CliniMACS Reagents to the Cell Preparation Bag
Selection buffer	See CliniMACS PBS/EDTA Buffer.
Selection column	See separation column.
Selection column holder	See separation column holder.
Separation column	Column in which magnetically labeled cells are separated when exposed to the magnetic field
Separation column holder	Molded guides in the magnet housing that holds the separation column in place
Separation program	Software program designed for the enrichment or depletion of magnetically labeled cell subsets from a mixed cell population. Choose from a menu of separation programs depending on the intended separation.

Separation reagent	Reagent for magnetic labeling of cells, e.g., CliniMACS CD34 Reagent
T-fitting	T-shaped fitting on a tubing set where three tubing meet
TCRα/β antigen	The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β antigen is expressed on the majority of peripheral blood T cells.
Transfer bag	Bag with a tubing and a spike at the end
Wash Waste Bag	Collection bag in which the wash supernatant is collected by separation from the sedimented cell suspension after centrifugation steps during sample preparation
WBC	White blood cells

3 Warnings and precautions

This chapter explains the potential risks associated with the operation of the CliniMACS Plus System. At all times, local working area safety instructions, policies, standards regarding good manufacturing practice, health, safety, and prevention of accidents must be adhered to. For instructions for use, e.g., warnings and precautions, concerning the handling of biohazardous materials and cellular starting product, as well as the CliniMACS Plus System components, refer to the relevant sections in the instructions for use of the respective component. Detailed instructions for use regarding the CliniMACS Plus Instrument are described in the CliniMACS Plus Instrument User Manual.

Any serious incident that has occurred in relation to this product should be reported to Miltenyi Biotec B.V. & Co. KG – using the contact information provided – and the competent authority of the member state in which the user of this product is established.

3.1 Process related

⚠ WARNING

Risk of process failure or damage to the instrument. Risk of process failure or damage to the instrument if procedures are performed by untrained operators. All processing procedures must be performed by trained operators only. Operator training will be provided by a qualified Miltenyi Biotec representative.

⚠ CAUTION

Risk of reduced process performance. Temperatures lower or higher than room temperature may result in less viability, purity and yield of the target cells. Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

⚠ CAUTION

Equipment safety may be compromised. Equipment safety may be compromised if the instrument is not used according to the manufacturer's instructions. Fully read and understand the safety information, warnings, precautions, and instructions for proper operation of the CliniMACS Plus System provided in the CliniMACS Plus instructions for use and in any safety-related recommendations issued by Miltenyi Biotec.

- For the manufacturing and use of target cells in humans the national legislation and regulations – e.g., for the EU the Directive 2004/23/EC (human tissues and cells) or the Directive 2002/98/EC (human blood and blood components) – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS Plus System.
- All materials which have come into contact with blood and blood products, must be treated as infectious material. Regulations for the handling of infectious material must be observed.

- All tubing, fittings, valves, the pre-column, and the separation column should be checked thoroughly for leaks during the priming step.
- All bags, including those used in sample preparation, should be preserved until final analysis of the collected cells has been completed and successful separation of the target cells has been confirmed.

3.2 CliniMACS Reagents and Biotin Conjugates

- When the target cell fraction is infused or injected into patients, they may receive traces of murine antibody and iron-dextran.
- Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.
- The separation of cells using the CliniMACS Reagents and Biotin Conjugates must be performed by trained operators only.
- Operators using the CliniMACS Reagents and Biotin Conjugates should have experience in the separation of cells from bone marrow or peripheral blood.
- The non-target cells are not intended for therapeutic use and may not be reinfused into patients.
- The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients.
- The CliniMACS Reagents and Biotin Conjugates are not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- Do not use after the use-by date printed on the product label.
- Do not use if package is damaged. Use reagent only if vial is undamaged and sealed.

3.3 Handling of biohazardous material

⚠ WARNING

Risk of severe injury or death. Depending on the biological material used, contact may lead to severe personal injury or death. Always wear personal safety equipment in accordance with warnings and precautions, in particular if biohazardous material is or has been used.

- To avoid contamination of the cellular starting product, all preparation steps should be performed under sterile conditions using aseptic techniques (e.g., in the laminar flow hood).
- The operator performing the cell separation must be trained in the proper use of the equipment and in the handling of blood products and bone marrow aspirate.
- The operator performing the cell separation should wear appropriate clothing (e.g., lab coat, gloves and eyeglasses or goggles) when working with patient samples and handling potentially biohazardous material.
- All blood products must be treated as a potential biohazard. Leukapheresis product, blood product, bone marrow aspirate, collected cells, used buffer, used tubing set and other materials that have been in contact with these fluids must be treated as biohazardous materials according to standard hospital or institutional requirements.
- The CliniMACS Plus Instrument should be considered a potential biohazard after each separation run and cleaned with an aqueous biocidal detergent (e.g., Bacillol® plus or Meliseptol®, see also section “Cleaning and maintenance” in the CliniMACS Plus Instrument User Manual) according to standard hospital or institutional requirements.
- Disposable materials must be treated as biohazardous materials according to standard hospital or institutional requirements.

3.4 Cellular starting product

IMPORTANT

Labeling and separation of cells should begin as soon as possible after the cellular starting product has been collected. The product should not be older than 24 hours when starting the labeling and separation procedure.

- The cellular starting product (e.g., leukapheresis product) should be collected according to standard hospital or institutional procedures in standard collection bags.
- The container containing the cellular starting product should be labeled with patient identification, time, date and place of collection according to procedures specified for use with the clinical protocol.
- For transportation, the cellular starting product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. Do not refrigerate. The cell concentration should not exceed 0.2×10^9 cells per mL during transportation.
- Avoid intensive mixing of the cellular starting material.
- If the cellular starting product has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). During storage, the concentration of leukocytes should never exceed 0.2×10^9 cells per mL.
- Cells should be stored in autologous plasma. If the cell concentration is higher than 0.2×10^9 cells per mL, dilute the cellular starting product with autologous plasma.

4 The CliniMACS Plus System

The CliniMACS Plus System, consisting of the components CliniMACS Plus Instrument, CliniMACS Reagent(s), CliniMACS Tubing Set, CliniMACS PBS/EDTA Buffer, and further accessories, which must be used in combination, is intended for the *in vitro* separation of specific human cells for clinical applications.

IMPORTANT
For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Plus System components, refer to the instructions for use provided for the respective component.

4.1 CliniMACS Plus Instrument

The CliniMACS Plus Instrument is an electromechanical device incorporating a permanent magnet, a peristaltic pump, pinch valves, electronics and software.

4.2 CliniMACS Reagents and Biotin Conjugates

The CliniMACS Reagents are dark colored, non-viscous, colloidal solutions, containing the cell specific antibody conjugates in buffer. The reagents consist of the antibody chemically coupled to super-paramagnetic particles.

The CliniMACS Biotin Conjugates are clear and colorless solutions containing antibody covalently linked to biotin in buffer. The antibodies are highly specific, making *in vitro* labeling of rare target cells possible.

4.3 CliniMACS Tubing Sets

The CliniMACS Tubing Sets are sterile, single-use disposables designed to be used in combination with the CliniMACS Plus Instrument for the *in vitro* enrichment or depletion of human cells from heterogeneous haematologic cell populations.

4.4 CliniMACS PBS/EDTA Buffer

The CliniMACS PBS/EDTA Buffer is a wash and transport fluid to enable the *in vitro* separation of human cells.

4.5 CliniMACS Accessories

- **plasma transfer set:**
  transfer set with two spikes for plasma or fluids, Transfer Set Coupler/Coupler (REF 200-073-902), or equivalent
- **Luer/Spike Interconnector:**
  transfer set with spike and syringe adapter (female luer) for plasma or fluids, Luer/Spike Interconnector (REF 200-073-903), or equivalent

4.6 Additional materials required

In addition to the CliniMACS Products, additional materials may be required for a CliniMACS Plus Separation:

- **cell culture bags, gas-permeable:**
  MACS GMP Cell Differentiation Bag – 100 mL (REF 170-076-400), or equivalent
- **transfer bags, suitable for centrifugation:**
  Transfer bag 150 mL, Terumo, or equivalent
  Transfer bag 600 mL, Terumo , or equivalent
  Transfer bag 1,000 mL, Terumo , or equivalent
- **sampling site coupler:**
  Sampling site coupler, Terumo, or equivalent
- **pre-system filter:**
  Blood Transfusion Filter, Haemonetics®, or equivalent
- **syringes and needles:**
  appropriate syringes (5 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20-gauge needles
- **locking forceps**
- **sample tubes**
- **human serum albumin (HSA):**
  Use HSA of pharmaceutical grade quality only, which is available as approved product in the country of the user.
- **clinical grade immunoglobulin G:**
  Use IgG of pharmaceutical grade quality only, which is available as approved drug in the country of the user, e. g., for Germany: Gamunex® 10%, or equivalent.
- **cell culture medium:**
  e.g., RPMI 1640, or equivalent
- **application-specific materials:**
  individual biotinylated cell specific antibody or ligand; AB serum or, alternatively, autologous serum

4.7 Equipment required

- **uninterruptable power source:**
  recommended UPS: APC Smart-UPS 1500VA USB & Serial 230 V, manufactured by APC (American Power Conversion), or equivalent
- **laminar flow hood**
- **sterile tubing connector:**
  Terumo Sterile Connection Device (TSCD®), or equivalent
- **orbital rotator:**
  Platform shakers, Heidolph Instruments, or equivalent
- **centrifuge:**
  Centrifuge, Sorvall, or equivalent, and buckets for centrifugation with aerosol containment caps
- **plasma extractor:**
  Plasma Separation Stand, Terumo Equipment, or equivalent
- **table top balance:**
  Laboratory Balance, Mettler Toledo, or equivalent, with 1 kg capacity; resolution to 0.1 g
- **tubing heat sealer:**
  Hematron III hand held sealer, Baxter, or equivalent
- **tubing stripper:**
  Tube Stripper, Baxter, or equivalent
- **biohazard waste containers**

Additional equipment required for the CliniMACS Cytokine Capture System (IFN-gamma):

- 24 well culture dish Corning™ Costar™ 24 well culture plate, Sigma Aldrich, or equivalent
- MiniMACS™ Separation Unit, Miltenyi Biotec (REF 130-042-102)
- MS Columns, Miltenyi Biotec (REF 130-042-201)
- Pre-Separation Filter, Miltenyi Biotec (REF 130-041-407)
- MACSmix™ Tube Rotator, Miltenyi Biotec (REF 130-090-753)

5 Overview of applications

5.1 Four STEPS to your target cells

In the following chapter an overview of the CliniMACS Plus Separation is presented. The CliniMACS Plus Separation is carried out in four STEPS. In the first step, the cells are prepared and magnetically labeled. In the second step, a suitable separation program is chosen. In a third step the tubing set is installed onto the CliniMACS Plus Instrument and in the fourth step cells are separated automatically.

Recording of application-related process data may be required. For supporting worksheets, contact Miltenyi Biotec Technical Support.

The CliniMACS Plus Applications User Manual comprises different options for each step (different reagents, tubing sets, and programs). Before starting an application, the application-specific chapters (chapter 6) and detailed explanation of STEPS 1–4 (chapter 7) must be chosen by using the overview table beginning on the next page. The overview table contains information on the applicable sections of the user manual. The overview table is based on the kind and number of reagents, the number of cells, the type of tubing set and the relevant separation program needed for the separation.

STEP 1: Cell preparation and magnetic labeling

STEP 1 describes the preparation of the cell product and the magnetic labeling of the specific cells, expressing the respective antigen. Follow the instructions of the applicable section. Continue with STEP 2.

STEP 2: Choice of separation program

STEP 2 describes the selection of a separation program of the CliniMACS Plus Instrument. Depending on cell type and cell number to be separated, different programs must be selected. To determine which program is applicable, go to the overview table on the next page. Read the respective section for the selected separation program. Follow the instructions given in the applicable section. Continue with STEP 3.

STEP 3: Installation of CliniMACS Tubing Sets

STEP 3 explains the installation of the tubing set onto the CliniMACS Plus Instrument. Follow the instructions in the applicable section. Continue with STEP 4.

STEP 4: CliniMACS Plus Separation

STEP 4 describes the automated CliniMACS Plus Separation. The CliniMACS Plus Instrument performs the automated cell separation procedure (chosen in STEP 2). Follow the instructions of the applicable section.

IMPORTANT

The overview table on the following pages summarizes information on typical applications of the CliniMACS Reagents. When separation of a different number of target cells is to be performed, refer to the instructions in the application-specific overview to find information on the maximum capacity of the respective application. Follow the instructions of the application-specific sections and detailed explanations of STEPS 1–4. See overview table.

5.2 Overview table

Application capacity	Reagent vial(s)	Tubing set	Separation program	Application-specific section	Detailed explanation of STEPS 1–4 sections			
					STEP 1	STEP 2	STEP 3	STEP 4
Anti-Biotin (REF 173-01) – Enrichment								
≤40×10^9 total cells	1× Anti-Biotin Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 1.1	6.3	7.1	7.2.3	7.3.1	7.4.2
Anti-Biotin (REF 173-01) – Depletion (normal-scale application)								
≤40×10^9 total cells	1× Anti-Biotin Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.3	7.1	7.2.5	7.3.1	7.4.4
Anti-Biotin (REF 173-01) – Depletion (large-scale application)								
>40–80×10^9 total cells	2× Anti-Biotin Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.3	7.1	7.2.5	7.3.1	7.4.4
CCS (REF 279-01) – Enrichment								
IFN-secreting cells from ≤1×10^9 total cells	1 kit (2 reagent vials)	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 3.2	6.17	7.1	7.2.4	7.3.1	7.4.3
CD1c (BDCA-1)-Biotin (REF 277-01) – Depletion - Part 1 of 2								
≤5×10^9 CD19 positive cells from ≤40×10^9 total cells (WBC)	1× CD19 Reagent and 1× CD1c (BDCA-1)-Biotin	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.12	7.1	7.2.5	7.3.1	7.4.4
CD1c (BDCA-1)-Biotin (REF 277-01) – Enrichment - Part 2 of 2								
≤0.2×10^9 CD1c (BDCA-1) positive cells from ≤40×10^9 total cells (WBC)	1× Anti-Biotin Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 3.2	6.13	7.1	7.2.4	7.3.1	7.4.3
CD3 (REF 273-01) – Depletion (normal-scale application)								
≤15×10^9 CD3 positive cells from ≤40×10^9 total cells	1× CD3 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.5	7.1	7.2.5	7.3.1	7.4.4
CD3 (REF 273-01) – Depletion (large-scale application)								
>15–20×10^9 CD3 positive cells from >40–80×10^9 total cells	2× CD3 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.5	7.1	7.2.5	7.3.1	7.4.4
CD3 (REF 273-01) – Depletion (normal-scale application)								
≤15×10^9 CD3 positive cells from ≤40×10^9 total cells	1× CD3 Reagent	1× CliniMACS Depletion Tubing Set (REF 261-01)	DEPLETION 3.1	6.5	7.1	7.2.6	7.3.3	7.4.5

Application capacity	Reagent vial(s)	Tubing set	Separation program	Application-specific section	Detailed explanation of STEPS 1–4 sections			
					STEP 1	STEP 2	STEP 3	STEP 4
CD3 (REF 273-01) – Depletion (large-scale application)								
>15–30×10^9 CD3 positive cells from >40–80×10^9 total cells	2× CD3 Reagent	1× CliniMACS Depletion Tubing Set (REF 261-01)	DEPLETION 3.1	6.5	7.1	7.2.6	7.3.3	7.4.5
CD4 (REF 276-01) – Enrichment								
≤5×10^9 CD4 positive cells from ≤40×10^9 total cells	1× CD4 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 1.1	6.10	7.1	7.2.3	7.3.1	7.4.2
CD4 (REF 276-01) – Depletion								
≤12×10^9 CD4 positive cells from ≤40×10^9 total cells	1× CD4 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.10	7.1	7.2.5	7.3.1	7.4.4
CD8 (REF 275-01) – Enrichment								
≤4×10^9 CD8 positive cells from ≤40×10^9 total cells	1× CD8 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 1.1	6.8	7.1	7.2.3	7.3.1	7.4.2
CD8 (REF 275-01) – Depletion								
≤4×10^9 CD8 positive cells from ≤40×10^9 total cells	1× CD8 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.8	7.1	7.2.5	7.3.1	7.4.4
CD4/CD8 combination (REF 276-01/REF 275-01) – Depletion								
≤12×10^9 CD4 positive cells and ≤4×10^9 CD8 positive cells from ≤40×10^9 total cells	1× CD4 Reagent and 1× CD8 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.11	7.1	7.2.5	7.3.1	7.4.4
CD14 (REF 272-01) – Enrichment								
≤4×10^9 CD14 positive cells from ≤20×10^9 total cells	1× CD14 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 1.1	6.2	7.1	7.2.3	7.3.1	7.4.2
CD19 (REF 179-01) – Depletion								
≤5×10^9 CD19 positive cells from ≤40×10^9 total cells	1× CD19 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.6	7.1	7.2.5	7.3.1	7.4.4
CD3/CD19 combination (REF 273-01/REF 179-01) – Depletion								
≤15×10^9 CD3 positive cells and ≤5×10^9 CD19 positive cells from ≤40×10^9 total cells	1× CD3 Reagent and 1× CD19 Reagent	1× CliniMACS Depletion Tubing Set (REF 261-01)	DEPLETION 3.1	6.7	7.1	7.2.6	7.3.3	7.4.5
CD25 (REF 274-01) – Enrichment								
≤0.6×10^9 CD25 positive cells from ≤40×10^9 total cells (WBC)	1× CD25 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 3.2	6.9	7.1	7.2.4	7.3.1	7.4.3

Application capacity	Reagent vial(s)	Tubing set	Separation program	Application-specific section	Detailed explanation of STEPS 1–4 sections			
					STEP 1	STEP 2	STEP 3	STEP 4
CD25 (REF 274-01) – Enrichment								
≤0.6×10^9 highly expressing CD25 positive cells from ≤40×10^9 total cells (WBC)	1× CD25 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 3.2	6.9	7.1	7.2.4	7.3.1	7.4.3
CD25 (REF 274-01) – Depletion (normal-scale application)								
≤6×10^9 CD25 positive cells from ≤40×10^9 total cells (WBC)	1× CD25 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.9	7.1	7.2.5	7.3.1	7.4.4
CD25 (REF 274-01) – Depletion (large-scale application)								
>6–12×10^9 CD25 positive cells from >40–80×10^9 total cells (WBC)	2× CD25 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	DEPLETION 2.1	6.9	7.1	7.2.5	7.3.1	7.4.4
CD34 (REF 171-01) – Enrichment (normal-scale application)								
≤0.6×10^9 CD34 positive cells from ≤60×10^9 total cells	1× CD34 Reagent	1× CliniMACS Tubing Set (REF 161-01)	CD34 SELECTION 1	6.1	7.1	7.2.2	7.3.1	7.4.1
CD34 (REF 171-01) – Enrichment (large-scale application)								
>0.6–1.2×10^9 CD34 positive cells from >60–120×10^9 total cells	2× CD34 Reagent	1× CliniMACS Tubing Set LS (REF 162-01)	CD34 SELECTION 2	6.1	7.1	7.2.2	7.3.1	7.4.1
CD45RA (REF 701-46) – Depletion								
≤20×10^9 CD45RA positive cells from ≤50×10^9 total cells	1× CD45RA Reagent	1× CliniMACS Depletion Tubing Set (REF 261-01)	DEPLETION 3.1	6.16	7.1	7.2.6	7.3.3	7.4.5
CD56 (REF 271-01) – Enrichment								
≤5×10^9 CD56 positive cells from ≤40×10^9 total cells	1× CD56 Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 1.1	6.4	7.1	7.2.3	7.3.1	7.4.2
CD304 (BDCA-4) (REF 278-01) – Enrichment								
≤0.2×10^9 CD304 (BDCA-4) positive cells from ≤40×10^9 total cells	1× CD304 (BDCA-4) Reagent	1× CliniMACS Tubing Set (REF 161-01)	ENRICHMENT 3.2	6.14	7.1	7.2.4	7.3.1	7.4.3
TCRα/β-Biotin (REF 701-48/REF 173-01) – Depletion								
≤24×10^9 TCRα/β positive cells from ≤60×10^9 total cells	1× TCRα/β-Biotin and 2× Anti-Biotin Reagent	1× CliniMACS Depletion Tubing Set (REF 261-01)	DEPLETION 3.1	6.15	7.1	7.2.6	7.3.3	7.4.5

Table 5.1: Overview of available applications with corresponding tubing sets and separation programs

6 Application-specific overview of STEPS 1–4

6.1 Enrichment of CD34 positive cells

The CliniMACS Plus CD34 System including the CliniMACS Plus Instrument, the CliniMACS CD34 Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

6.1.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- Normal-scale application: up to 0.6×10^9 CD34 positive cells out of up to 60×10^9 total cells (WBC)
- Large-scale application: greater than 0.6×10^9 and up to 1.2×10^9 CD34 positive cells out of up to 120×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalog number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	–
CliniMACS CD34 Reagent	171-01	• 1 vial (normal-scale) • 2 vials (large-scale)	–
CliniMACS Tubing Set	161-01	1 tubing set	CD34 SELECTION 1
CliniMACS Tubing Set LS	162-01	1 tubing set	CD34 SELECTION 2
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]	–

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.1: CliniMACS Materials required for enrichment of CD34 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bags:**
  three 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set
- one pre-system filter
- locking forceps
- appropriate syringes (different sizes) and hypodermic 20 gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

If the weight of the cellular starting product is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD34 positive cells, centrifuge the sample to reduce the volume to 200 g at the most and then proceed with following steps.

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ **Volume adjustment: labeling volume 95 g (±5 g) (normal-scale application) or 190 g (±5 g) (large-scale application)**

1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cell product – Labeling volume
2. Resuspend the cell pellet.

▶ **Incubation with the CliniMACS CD34 Reagent**

1. Add 1 or 2 vial(s) of CliniMACS CD34 Reagent (see Table 6.1). Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ **Removal of excess reagent**

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (200×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than these values, a total of three washing steps (instead of only two) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5 Fill Cell Preparation Bag with buffer.
6. Centrifuge (200×g, without brake, 15 minutes, RT).
7. Remove supernatant as much as possible.
8. Resuspend the cell pellet.
9. Adjust sample loading volume to 150 g (normal-scale application) or 275 g (large-scale application).

Note: Take a sample after removal of excess reagent for later analysis (optional).

6.1.2 STEP 2: Choice of separation program

▶ **Switch-on the CliniMACS Plus Instrument**

▶ **Choice of separation program CD34 SELECTION 1/2**
(using 1 vial: CD34 SELECTION 1, or using 2 vials: CD34 SELECTION 2)

See section 7.2.2 on page 131.

6.1.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.1.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.1 on page 184.

6.2 Enrichment of CD14 positive cells

The CliniMACS Plus CD14 System including the CliniMACS Plus Instrument, the CliniMACS CD14 Reagent, the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD14 positive cells from heterogeneous hematologic cell populations.

6.2.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 4×10^9 CD14 positive cells out of up to 20×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD14 Reagent	272-01	1 vial
CliniMACS Tubing Set	161-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]

1 See Table 6.3 and Table 6.4. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.2: CliniMACS Materials required for enrichment of CD14 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL or one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.4)
- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  two 1,000 mL transfer bags in combination with two Luer/Spike Interconnector to connect the bags to the tubing set (see Table 6.4)
- **If more than one liter of buffer is required for the separation:**
  one plasma transfer set to connect the buffer bags (see Table 6.4)
- one pre-system filter
- locking forceps
- appropriate syringes (different sizes) and hypodermic 20 gauge needles

Overview of materials required

Cell preparation	
Total cells (WBC)	≤20×10^9
Labeled cells	≤4×10^9
Number of reagent vials	1–2
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.3: Material required for cell preparation of CD14 positive cells

Separation		
Labeled cells	≤2×10^9	>2–4×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Plasma transfer set	–	1

Table 6.4: Material required for enrichment of CD14 positive cells

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

▶ Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 50 g (±5 g)

1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cellular starting product – Labeling volume
2. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD14 Reagent

1. Add 1 vial of CliniMACS CD14 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 15 minutes at RT.

- **Removal of excess reagent**

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

Note: Take a sample after removal of excess reagent for later analysis (optional).

6.2.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 1.1**
- **Sample parameter input**

See section 7.2.3 on page 133.

6.2.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.2.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.2 on page 188.

6.3 Enrichment/depletion with the CliniMACS Anti-Biotin Reagent

The CliniMACS Plus Anti-Biotin System including the CliniMACS Plus Instrument, the CliniMACS Anti-Biotin Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human cells previously labeled with biotinylated antibodies.

6.3.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- **Enrichment:** up to 40×10^9 total cells (WBC)
- **Depletion:**
  Normal-scale application: up to 40×10^9 total cells (WBC)
  Large-scale application: greater than 40×10^9 and up to 80×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	–
CliniMACS Anti-Biotin Reagent	173-01	• 1 vial (normal-scale) • 2 vials (large-scale)	–
CliniMACS Tubing Set	161-01	1 tubing set	ENRICHMENT 1.1
CliniMACS Tubing Set LS	162-01	1 tubing set	DEPLETION 2.1
CliniMACS PBS/EDTA Buffer	700-25	• 3×1,000 mL bags[1] (normal-scale) • 4×1,000 mL bags[1] (large-scale)	–

1 The amount of buffer varies depending on the selected separation program and the number of labeled cells (see Table 6.6 to Table 6.9). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.5: CliniMACS Materials required for enrichment/depletion with the CliniMACS Anti-Biotin Reagent

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 600 mL or one 1,000 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.7 and Table 6.9)
- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary:**
  two to three 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.7 and Table 6.9). Replace the Priming Waste Bag using the sterile tubing connector.
- **If more than one liter of buffer is required for the separation:**
  one to two plasma transfer sets to connect the buffer bags (see Table 6.7 and Table 6.9)
- one pre-system filter
- locking forceps
- appropriate syringes (different sizes) and hypodermic 20 gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes
- individual biotinylated cell-specific antibody or ligand

Overview of materials

Cell preparation (ENRICHMENT 1.1)	
Total cells (WBC)	≤40×10^9
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	2,000 mL
Plasma transfer set	2

Table 6.6: Materials required for cell preparation (ENRICHMENT 1.1)

Separation (ENRICHMENT 1.1)		
Labeled cells	≤2×10^9	>2–5×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Plasma transfer set	–	1

Table 6.7: Materials required for enrichment (ENRICHMENT 1.1)

Cell preparation (DEPLETION 2.1)		
Total cells (WBC)	≤40×10^9	>40–80×10^9
Number of reagent vials	1	2
CliniMACS PBS/EDTA Buffer	2,000 mL	2,000 mL
Plasma transfer set	2	2

Table 6.8: Materials required for cell preparation (DEPLETION 2.1)

Separation (DEPLETION 2.1)			
Labeled cells	<5×10^9	5–13×10^9	>13–20×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–		
Priming Waste Bag	–	–	
Cell Collection Bag	600 mL	1,000 mL	1,000 mL
Luer/Spike Interconnector	1	3	4
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL	3,000 mL
Plasma transfer set	–	1	2

Table 6.9: Materials required for depletion (DEPLETION 2.1)

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

Centrifugation

1. Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
2. Remove supernatant to adjust volume for primary antibody labeling as recommended by the manufacturer of the primary antibody.
3. Resuspend the cell pellet.

Labeling of the cells: incubation with the primary antibody

Follow the instructions for use provided by the manufacturer of the primary antibody.

Removal of excess conjugate (1st wash)

1. Add buffer: Weight of buffer to be added = 600g – Weight of cell product
2. Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

Volume adjustment: labeling volume 95 g (±5 g) (normal-scale application) or 190 g (±5 g) (large-scale application)

1. Remove supernatant to adjust the sample using the following equation: eight of supernatant to be removed = Weight of diluted cell product – Labeling volume
   Note: Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
2. Resuspend the cell pellet.

Magnetic labeling of the cells: incubation with the CliniMACS Anti-Biotin Reagent

1. Add 1 or 2 vial(s) of CliniMACS Anti-Biotin Reagent (see Table 6.6 and Table 6.8). Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

- **Removal of excess reagent (2nd wash)**

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than the value listed above, a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g (normal-scale application) or 200 g (large-scale application).

Note: Take a sample after removal of excess reagent for later analysis (optional).

6.3.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1**
- **Sample parameter input**

See section 7.2.3 on page 133 or 7.2.5 on page 140.

6.3.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.3.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.2 on page 188 and 7.4.4 on page 196.

6.4 Enrichment of CD56 positive cells

The CliniMACS Plus CD56 System including the CliniMACS Plus Instrument, the CliniMACS CD56 Reagent, the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD56 positive cells from heterogeneous hematologic cell populations.

6.4.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 5×10^9 CD56 positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD56 Reagent	271-01	1 vial
CliniMACS Tubing Set	161-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]

1 See Table 6.11 and Table 6.12. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.10: CliniMACS Materials required for enrichment of CD56 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL or 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.12)

- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  two 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.12)
- **If more than one liter of buffer is required for the separation:**
  one plasma transfer set to connect the buffer bags (see Table 6.12)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

Overview of materials required

Cell preparation	
Total cells (WBC)	≤40×10^9
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.11: Materials required for the cell preparation of CD56 positive cells

Separation		
Labeled cells	≤2×10^9	>2–5×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Plasma transfer set	–	1

Table 6.12: Materials required for the enrichment of CD56 positive cells

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = 95 g + Weight of empty CPB
4. Resuspend the cells.

▶ Incubation with the CliniMACS CD56 Reagent

1. Add 1 vial of CliniMACS CD56 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet carefully after removal of supernatant.
5. Adjust sample loading volume to 150 g.

Note: Take a sample after removal of excess reagent for later analysis (optional).

6.4.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 1.1**
- **Sample parameter input**

See section 7.2.3 on page 133.

6.4.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.4.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.2 on page 188.

6.5 Depletion of CD3 positive cells

The CliniMACS Plus CD3 System including the CliniMACS Plus Instrument, the CliniMACS CD3 Reagent, the CliniMACS Tubing Set LS or the CliniMACS Depletion Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD3 positive cells from heterogeneous hematologic cell populations.

6.5.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- Normal-scale application: up to 15×10^9 CD3 positive cells out of up to 40×10^9 total cells (WBC) using either the CliniMACS Tubing Set LS or the CliniMACS Depletion Tubing Set
- Large-scale application: greater than 15×10^9 and up to 20×10^9 CD3 positive cells out of greater than 40×10^9 and up to 80×10^9 total cells (WBC) using the CliniMACS Tubing Set LS, or greater than 15×10^9 and up to 30×10^9 CD3 positive cells out of greater than 40×10^9 and up to 80×10^9 total cells (WBC) using the CliniMACS Depletion Tubing Set

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	
CliniMACS CD3 Reagent	273-01	• 1 vial (normal-scale) • 2 vials (large-scale)	
CliniMACS Tubing Set LS	162-01	1 tubing set	DEPLETION 2.1
CliniMACS Depletion Tubing Set	261-01	1 tubing set	DEPLETION 3.1
CliniMACS PBS/EDTA Buffer	700-25	Up to 5×1,000 mL bags[1]	

1 See Table 6.14 to Table 6.16. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

2 **Note:** For high numbers of labeled cells it is recommended to use a CliniMACS Depletion Tubing Set, in order to save processing time (e.g., 20×10^9 labeled cells will be processed within five hours using the CliniMACS Tubing Set LS versus two hours using a CliniMACS Depletion Tubing Set).

Table 6.13: CliniMACS Materials required for depletion of CD3 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set

  If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags. One 600 mL transfer bag is already attached to the CliniMACS Depletion Tubing Set (see Table 6.15 and Table 6.16).
- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary:**
  two to three 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.15 and Table 6.16)

  Replace the Priming Waste Bag using the sterile tubing connector.
- **If more than one liter buffer is required for the separation:**
  one to two plasma transfer sets to connect the buffer bags (see Table 6.15 and Table 6.16)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- clinical grade immunoglobulin G
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Reapplication Bag, Cell Collection Bag, Non-Target Cell Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags is necessary.

Overview of materials required

Cell preparation		
Total cells (WBC)	≤40×10^9	>40–80×10^9
Labeled cells	≤15×10^9	>15–30×10^9
Number of reagent vials	1	2
CliniMACS PBS/EDTA Buffer	2,000 mL	2,000 mL
Plasma transfer set	2	2
Sample loading volume	150 mL	250–300 mL

Table 6.14: Materials required for the cell preparation of CD3 positive cells

Separation (DEPLETION 2.1)			
Labeled cells	≤5×10^9	>5–13×10^9	>13–20×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–		
Priming Waste Bag	–	–	
Cell Collection Bag	600 mL	1–2×600 mL	2×600 mL
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL	3,000 mL
Luer/Spike Interconnector	1	3	3
Plasma transfer set	–	1–2	3

Table 6.15: Materials required for the depletion of CD3 positive cells (DEPLETION 2.1)

Separation (DEPLETION 3.1)		
Labeled cells	≤29×10^9	>29–30×10^9
Non-Target Cell Bag	No bag replacement necessary	
Buffer Waste Bag		
Reapplication Bag		
Cell Collection Bag	–	1×600 mL (additionally)
CliniMACS PBS/EDTA Buffer	1,000 mL	
Plasma transfer set	–	1

Table 6.16: Materials required for the depletion of CD3 positive cells (DEPLETION 3.1)

Note: The materials required for the separation can also be determined by switching on the CliniMACS Plus Instrument, selecting the separation program to be used (DEPLETION 2.1 for the CliniMACS Tubing Set LS and DEPLETION 3.1 for the CliniMACS Depletion Tubing Set) and entering the sample parameters. The CliniMACS Plus Software will calculate the required sizes of bags to be connected to the tubing set and the required weight of buffer.

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

Determine the number of all CD3 labeled cells (including unspecific CD3 binding, not only real T cells). Consider this number for application specifications and sample parameter input (see STEP 2).

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g) (normal-scale application) or 190 g (±5 g) (large-scale application)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation (consider IgG weight): Target weight of filled CPB = Labeling volume – IgG weight + Weight of empty CPB
4. Resuspend the cell pellet.

▶ Incubation with clinical grade immunoglobulin

1. Add IgG (1.5 mg/mL).
2. Incubate on orbital rotator (25 rpm) for 5 minutes at RT.

▶ Incubation with the CliniMACS CD3 Reagent

1. Add 1 or 2 vial(s) of CliniMACS CD3 Reagent (see Table 6.14). Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

- **Removal of excess reagent**

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 150 g (normal-scale application) or 250–300 g (large-scale application).

6.5.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program DEPLETION 2.1 or DEPLETION 3.1**
  (while using CliniMACS Tubing Set LS or CliniMACS Depletion Tubing Set, respectively)
- **Sample parameter input**
  (according to analysis of cellular starting product (see STEP 1))

See section 7.2.5 on page 140 or 7.2.6 on page 145.

6.5.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

or

- **CliniMACS Depletion Tubing Set**

See section 7.3.1 on page 150 or 7.3.3 on page 170.

6.5.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.4 on page 196 and 7.4.5 on page 200.

6.6 Depletion of CD19 positive cells

The CliniMACS Plus CD19 System including the CliniMACS Plus Instrument, the CliniMACS CD19 Reagent, the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD19 positive cells from heterogeneous hematologic cell populations.

6.6.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 5×10^9 CD19 positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD19 Reagent	179-01	1 vial
CliniMACS Tubing Set LS	162-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	2×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.17: CliniMACS Materials required for depletion of CD19 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set
- one pre-system filter
- locking forceps

- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 "STEP 1: Cell preparation and magnetic labeling" on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = 95 g + Weight of empty CPB
4. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD19 Reagent

1. Add 1 vial of CliniMACS CD19 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

- **Removal of excess reagent**

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.6.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program DEPLETION 2.1**
- **Sample parameter input**

See section 7.2.5 on page 140.

6.6.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.6.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.4 on page 196.

6.7 Depletion of CD3 positive cells and CD19 positive cells

The CliniMACS Plus CD3/CD19 System including the CliniMACS Plus Instrument, the CliniMACS CD3 Reagent, the CliniMACS CD19 Reagent, CliniMACS Depletion Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the simultaneous *in vitro* depletion of human CD3 positive cells and human CD19 positive cells from heterogeneous hematologic cell populations.

6.7.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 15×10^9 CD3 positive cells and up to 5×10^9 CD19 positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD3 Reagent	273-01	1 vial
CliniMACS CD19 Reagent	179-01	1 vial
CliniMACS Depletion Tubing Set	261-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	4×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.18: CliniMACS Materials required for depletion of CD3 positive cells and CD19 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- clinical grade immunoglobulin G
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Reapplication Bag, Cell Collection Bag, Non-Target Cell Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags required is necessary.

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

Determine the number of all CD3 and CD19 labeled cells (including unspecific CD3 and CD19 bindings, not only real T and B cells). Consider this number for application specifications and sample parameter input (see STEP 2).

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

Volume adjustment: labeling volume 87.5 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation (consider IgG weight): Target weight of filled CPB = 87.5g – IgG weight + Weight of empty CPB
4. Resuspend the cell pellet.

Incubation with clinical grade immunoglobulin

1. Add IgG (1.5 mg/mL).
2. Incubate on orbital rotator (25 rpm) for 5 minutes at RT.

Incubation with the CliniMACS CD3 Reagent and the CliniMACS CD19 Reagent

1. Add 1 vial of CliniMACS CD3 Reagent and 1 vial of CliniMACS CD19 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 150 g.

6.7.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program DEPLETION 3.1**
- **Sample parameter input**
  (according to analysis of cellular starting product (see STEP 1))

See section 7.2.6 on page 145.

6.7.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Depletion Tubing Set**

See section 7.3.3 on page 170.

6.7.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.5 on page 200.

6.8 Enrichment/depletion of CD8 positive cells

The CliniMACS Plus CD8 System including the CliniMACS Plus Instrument, the CliniMACS CD8 Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD8 positive cells from heterogeneous hematologic cell populations.

6.8.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- **Enrichment:** up to 4×10^9 labeled cells out of up to 40×10^9 total cells (WBC) using the CliniMACS Tubing Set
- **Depletion:** up to 4×10^9 labeled cells out of up to 40×10^9 total cells (WBC) using the CliniMACS Tubing Set LS

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	–
CliniMACS CD8 Reagent	275-01	1 vial	–
CliniMACS Tubing Set	161-01	1 tubing set	ENRICHMENT 1.1
CliniMACS Tubing Set LS	162-01	1 tubing set	DEPLETION 2.1
CliniMACS PBS/EDTA Buffer	700-25	Up to 3×1,000 mL bags[1]	–

1 See Table 6.20 to Table 6.22. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.19: CliniMACS Materials required for enrichment/depletion of CD8 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.21 and Table 6.22)
- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  two 1,000 mL transfer bags in combination with two Luer/ Spike Interconnectors to connect the bags to the tubing set (see Table 6.21 and Table 6.22)
- **If more than one liter of buffer is required for the separation:**
  one plasma transfer set to connect the buffer bags (see Table 6.21 and Table 6.22)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

Overview of materials required

Cell preparation	
Total cells	$\leq 40 \times 10^9$
Labeled cells	$\leq 4 \times 10^9$
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1
Sample loading volume	100 mL

Table 6.20: Materials required for the cell preparation of CD8 positive cells

Separation (ENRICHMENT 1.1)		
Labeled cells	<2×10^9	2–4×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	600 mL	600 mL
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	–	1

Table 6.21: Materials required for the enrichment of CD8 positive cells

Separation (DEPLETION 2.1)	
Labeled cells	≤4×10^9
Negative Fraction Bag	No bag replacement necessary
Buffer Waste Bag	
Priming Waste Bag	
Cell Collection Bag	600 mL
CliniMACS PBS/EDTA Buffer	1,000 mL
Luer/Spike Interconnector	1

Table 6.22: Materials required for the depletion of CD8 positive cells

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer. Calculate the target weight of the CPB filled with diluted cell product using the equation: Target weight of filled CPB = 95 g – Weight of empty CPB
4. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD8 Reagent

1. Add 1 vial of CliniMACS CD8 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.8.2 STEP 2: Choice of separation program

▶ Switch-on the CliniMACS Plus Instrument

▶ Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1

▶ Sample parameter input

See section 7.2.3 on page 133 or 7.2.5 on page 140.

6.8.3 STEP 3: Installation of CliniMACS Tubing Sets

▶ CliniMACS Tubing Set and CliniMACS Tubing Set LS

See section 7.3.1 on page 150.

6.8.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.2 on page 188 or 7.4.4 on page 196.

6.9 Enrichment/depletion of CD25 positive cells

The CliniMACS Plus CD25 System including the CliniMACS Plus Instrument, the CliniMACS CD25 Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD25 positive cells – preferably CD25 highly expressing cells – from heterogeneous hematologic cell populations.

6.9.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- **Enrichment:** CD25 positive or CD25 highly expressing cells: up to 0.6×10^9 labeled cells out of up to 40×10^9 total cells (WBC) using the CliniMACS Tubing Set
- **Depletion:**
  Normal-scale application: up to 6×10^9 CD25 positive cells out of up to 40×10^9 total cells (WBC) using the CliniMACS Tubing Set LS
  Large-scale application: greater than 6×10^9 and up to 12×10^9 CD25 positive cells out of greater than 40×10^9 and up to 80×10^9 total cells (WBC) using the CliniMACS Tubing Set LS

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	–
CliniMACS CD25 Reagent	274-01	• 1 vial (normal-scale) • 2 vials (large-scale)	–
CliniMACS Tubing Set	161-01	1 tubing set	ENRICHMENT 3.2
CliniMACS Tubing Set LS	162-01	1 tubing set	DEPLETION 2.1
CliniMACS PBS/EDTA Buffer	700-25	Up to 4×1,000 mL bags[1]	–

1 See Table 6.24 to Table 6.27. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.23: CliniMACS Materials required for enrichment/depletion of CD25 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one to two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL (or two 1,000 mL) transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL or one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set

  If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags (see Table 6.25 and Table 6.27).
- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  two 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.25 and Table 6.27)
- **If more than one liter of buffer is required for the separation:**
  one plasma transfer set to connect the buffer bags (see Table 6.25 and Table 6.27)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

Overview of materials required

Cell preparation (ENRICHMENT 3.2)		
Application	**Enrichment of CD25 positive cells**	**Enrichment of CD25 highly expressing cells**
Total cells (WBC)	≤40×10^9	≤40×10^9
Labeled cells	≤0.6×10^9	≤0.6×10^9
Number of reagent vials	1	1
CliniMACS PBS/EDTA Buffer	1,000–2,000 mL	2,000 mL
Plasma transfer set	1–2	2
Labeling volume	190 mL	380 mL
Labeling temperature	Room temperature	+4 °C to +8 °C (+39 °F to +46 °F)

Table 6.24: Materials required for the cell preparation of CD25 positive/highly expressing cells (ENRICHMENT 3.2)

Separation (ENRICHMENT 3.2)		
Application	**Enrichment of CD25 positive cells**	**Enrichment of CD25 highly expressing cells**
CliniMACS PBS/EDTA Buffer	1,000 mL	1,000 mL
Negative Fraction Bag		
Buffer Waste Bag	No bag replacement necessary	
Priming Waste Bag		
Cell Collection Bag	150 mL	150 mL
Luer/Spike Interconnector	1	1

Table 6.25: Materials required for enrichment CD25 positive/highly expressing cells (ENRICHMENT 3.2)

Cell preparation (DEPLETION 2.1)		
Total cells (WBC)	≤40×10^9	>40–80×10^9
Labeled cells	≤6×10^9	>6–12×10^9
Number of reagent vials	1	2
CliniMACS PBS/EDTA Buffer	1,000–2,000 mL	1,000–2,000 mL
Plasma transfer set	1–2	1–2
Labeling volume	95 mL	190 mL
Labeling temperature	Room temperature	Room temperature

Table 6.26: Materials required for the cell preparation of CD25 positive cells (DEPLETION 2.1)

Separation (DEPLETION 2.1)		
Total cells (WBC)	≤40×10^9	>40–80×10^9
CliniMACS PBS/EDTA Buffer	1,000 mL for <5×10^9 labeled cells	2,000 mL for 5–12×10^9 labeled cells
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	600 mL	2×600 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	–	2

Table 6.27: Materials required for the depletion of CD25 positive cells (DEPLETION 2.1)

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

IMPORTANT

- *Store the buffer for cell preparation for the enrichment or depletion of CD25 positive cells at +19 °C to +25 °C (+66 °F to +77 °F).*
- *Magnetic labeling during the cell preparation for the enrichment of CD25 highly expressing cells has to be performed using cold buffer (+4 °C to +8 °C [+39 °F to+46 °F]). Lower or higher temperature may result in less purity and yield of the target cells.*

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

Volume adjustment: labeling volume for

enrichment of CD25 positive cells: 190 g (±5 g)
enrichment of CD25 highly expressing cells: 380 g (±5 g)[1]
depletion of CD25 positive cells (normal-scale application): 95 g
depletion of CD25 positive cells (large-scale application): 190 g

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = labeling volume + Weight of empty CPB
4. Resuspend the cell pellet.

Incubation with the CliniMACS CD25 Reagent

1. Add 1 or 2 vial(s) of CliniMACS CD25 Reagent (see Table 6.24 and Table 6.26). Mix contents gently.
2. Incubate on orbital rotator (25 rpm):
   a) enrichment or depletion of CD25 positive cells: 30 minutes at RT
   b) enrichment of CD25 highly expressing cells: 15 minutes at +4 °C to +8 °C (+39 °F to +46 °F)

Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g (normal-scale application) or 200 g (large-scale application).

1 The magnetic labeling for the enrichment of CD25 highly expressing cells has to be performed using cold buffer (+4°C to +8°C [+39 °F to +46 °F]).

6.9.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 3.2 or DEPLETION 2.1**
- **Sample parameter input**
  (not for ENRICHMENT 3.2)

See section 7.2.4 on page 138 or 7.2.5 on page 140.

6.9.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.9.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.3 on page 192 or 7.4.4 on page 196.

6.10 Enrichment/depletion of CD4 positive cells

The CliniMACS Plus CD4 System including the CliniMACS Plus Instrument, the CliniMACS CD4 Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD4 positive cells from heterogeneous hematologic cell populations.

6.10.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

- **Enrichment:** up to 5×10^9 labeled cells out of up tp 40×10^9 total cells (WBC) using the CliniMACS Tubing Set
- **Depletion:** up to 12×10^9 labeled cells out of up to 40×10^9 total cells (WBC) using the CliniMACS Tubing Set LS

Note: If the number of calculated cells exceeds the application capacity, split the sample and continue with each sample separately, using a new tubing set for every CliniMACS Plus Instrument run.

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity	Separation program
CliniMACS Plus Instrument	151-01	1	–
CliniMACS CD4 Reagent	276-01	1 vial	–
CliniMACS Tubing Set	161-01	1 tubing set	ENRICHMENT 1.1
CliniMACS Tubing Set LS	162-01	1 tubing set	DEPLETION 2.1
CliniMACS PBS/EDTA Buffer	700-25	Up to 4×1,000 mL bags[1]	–

1 See Table 6.29 to Table 6.32. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.28: CliniMACS Materials required for enrichment/depletion of CD4 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL (or two 1,000 mL) transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL or one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set

  If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags (see Table 6.30 and Table 6.32).
- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary:**
  up to three 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.30 and Table 6.32)

  Replace the Priming Waste Bag using the sterile tubing connector.
- **If more than one liter of buffer is required for the separation:**
  one to two plasma transfer sets to connect the buffer bags (see Table 6.30 and Table 6.32)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

Overview of materials required

Cell preparation (ENRICHMENT 1.1)	
Total cells (WBC)	≤40×10^9
Labeled cells	≤5×10^9
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.29: Materials required for cell preparation of CD4 positive cells (ENRICHMENT 1.1)

Separation (ENRICHMENT 1.1)		
Labeled cells	≤2×10^9	>2–5×10^9
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	–	1

Table 6.30: Materials required for the enrichment of CD4 positive cells (ENRICHMENT 1.1)

Cell preparation (DEPLETION 2.1)	
Total cells (WBC)	≤40×10^9
Labeled cells	≤12×10^9
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.31: Materials required for cell preparation of CD4 positive cells (DEPLETION 2.1)

Separation (DEPLETION 2.1)		
Labeled cells	<5×10^9	5–12×10^9
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	1×600 mL	1–2×600 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	–	1–2

Table 6.32: Materials required for depletion of CD4 positive cells (DEPLETION 2.1)

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 "STEP 1: Cell preparation and magnetic labeling" on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cell product – 95 g
2. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD4 Reagent

1. Add 1 vial of CliniMACS CD4 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.10.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1**
- **Sample parameter input**

See section 7.2.3 on page 133 and 7.2.5 on page 140.

6.10.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.10.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.2 on page 188 or 7.4.4 on page 196.

6.11 Depletion of CD4 positive cells and CD8 positive cells

The CliniMACS Plus CD4/CD8 System including the CliniMACS Plus Instrument, the CliniMACS CD4 Reagent, the CliniMACS CD8 Reagent, the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the simultaneous *in vitro* depletion of human CD4 positive and CD8 positive cells from heterogeneous hematologic cell populations.

6.11.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 12×10^9 CD4 positive cells and up to 4×10^9 CD8 positive cells out of up to 40×10^9 total cells (WBC)

Note: If the number of calculated cells exceeds the application capacity, split the sample and continue with each sample separately, using a new tubing set for every CliniMACS Plus Instrument run.

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD4 Reagent	276-01	1 vial
CliniMACS CD8 Reagent	275-01	1 vial
CliniMACS Tubing Set LS	162-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	Up to 4×1,000 mL bags[1]

1 See Table 6.34 and Table 6.35. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.33: CliniMACS Materials required for depletion of CD4 positive cells and CD8 positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  two 600 mL (or two 1,000 mL) transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set

  If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bag (see Table 6.35).
- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary:**
  up to three 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.35)

  Replace the Priming Waste Bag using the sterile tubing connector.
- **If more than one liter of buffer is required for the separation:**
  one to two plasma transfer sets to connect the buffer bags (see Table 6.35)
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

IMPORTANT

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

Overview of materials required

Cell preparation	
Total cells (WBC)	≤40×10^9
Labeled cells	≤12×10^9 CD4 positive cells and ≤4×10^9 CD8 positive cells
Number of reagent vials	1 vial CliniMACS CD4 Reagent and 1 vial CliniMACS CD8 Reagent
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.34: Materials required for cell prepration of CD4 positive and CD8 positive cells

Separation			
Labeled cells	<5×10^9	5–13×10^9	>13–16×10^9
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL	3,000 mL
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–		
Priming Waste Bag	–	–	
Cell Collection Bag	1× 600 mL	1–2× 600 mL	2× 600 mL
Luer/Spike Interconnector	1	3	3
Plasma transfer set	–	1–2	3

Table 6.35: Materials required for depletion of CD4 positive and CD8 positive cells

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F])

▶ Volume adjustment: labeling volume 88 g (±5 g)

1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cell product – Labeling volume
2. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD4 Reagent and CliniMACS CD8 Reagent

1. Add 1 vial of CliniMACS CD4 Reagent and 1 vial of CliniMACS CD8 Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.11.2 STEP 2: Choice of separation program

▶ Switch-on the CliniMACS Plus Instrument

▶ Choice of separation program DEPLETION 2.1

▶ Sample parameter input

See section 7.2.5 on page 140.

6.11.3 STEP 3: Installation of CliniMACS Tubing Sets

▶ CliniMACS Tubing Set and CliniMACS Tubing Set LS

See section 7.3.1 on page 150.

6.11.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.4 on page 196.

6.12 Enrichment of CD1c (BDCA-1) positive cells – Part 1: Depletion of CD19 positive cells and labeling of CD1c (BDCA-1) positive cells

The CliniMACS Plus CD1c (BDCA-1)-Biotin System including the CliniMACS Plus Instrument, the CliniMACS CD1c (BDCA-1)-Biotin, the CliniMACS CD19 Reagent, the CliniMACS Anti-Biotin Reagent, the CliniMACS Tubing Set LS, the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD1c (BDCA-1) positive cells from heterogeneous hematologic cell populations after depletion of CD19 positive cells.

6.12.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Depletion of up to 5×10^9 CD19 positive cells and labeling of up to 0.2×10^9 CD1c (BDCA-1) positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD1c (BDCA-1)-Biotin	277-01	1 vial
CliniMACS CD19 Reagent	179-01	1 vial
CliniMACS Tubing Set LS	162-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	2×1,000 mL bags[1]

1 Before use, supplement buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.36: CliniMACS Materials required for enrichment of CD1c (BDCA-1) positive cells (Part 1)

Additional materials required (for Part 1 and Part 2)

- **Cell Preparation Bag:**
  two 600 mL transfer bags suitable for centrifugation as well as two sampling site couplers and three plasma transfer sets, for use during the cell preparation procedures
- **Plasma Waste Bags and Wash Waste Bags:**
  five 600 mL transfer bags suitable for centrifugation
- **Cell Collection Bag (Part 1):**
  one 600 mL transfer bag suitable for centrifugation in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set
- **Cell Collection Bag (Part 2):**
  one 150 mL transfer bag suitable for centrifugation in combination with a Luer/Spike Interconnector to connect the transfer bag to the tubing set
- two pre-system filters
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

Magnetic labeling of cells (Part 1)

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 90 g (±5 g)

1. Resuspend the cell pellet carefully after removal of supernatant.
2. Adjust the weight of the Cell Preparation Bag Part 1 (CPB) by adding buffer using the following equation: Target weight of filled CPB Part 1 = 90 g + Weight of empty CPB
3. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD19 Reagent and the CliniMACS CD1c (BDCA-1)-Biotin

1. Add 1 vial of CliniMACS CD19 Reagent and 1 vial of CliniMACS CD1c (BDCA-1)-Biotin. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag Part 1 with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.12.2 STEP 2: Choice of separation program

▶ Switch-on the CliniMACS Plus Instrument

▶ Choice of separation program DEPLETION 2.1

▶ Sample parameter input

See section 7.2.5 on page 140.

6.12.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.12.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.4 on page 196.

6.13 Enrichment of CD1c (BDCA-1) positive cells – Part 2: Flexible Labeling System

The CliniMACS Plus CD1c (BDCA-1)-Biotin System including the CliniMACS Plus Instrument, the CliniMACS CD1c (BDCA-1)-Biotin, the CliniMACS CD19 Reagent, the CliniMACS Anti-Biotin Reagent, the CliniMACS Tubing Set LS, the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD1c (BDCA-1) positive cells from heterogeneous hematologic cell populations after depletion of CD19 positive cells.

6.13.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 0.2×10^9 CD1c (BDCA-1) positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS Anti-Biotin Reagent	173-01	1 vial
CliniMACS Tubing Set	161-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]

1 Before use, supplement buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.37: CliniMACS Materials required for enrichment of CD1c (BDCA-1) positive cells (Part 2)

Additional materials required

Refer to part 1 for the additional materials required.

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells (CD19 depleted cell product (Part 2))

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Transfer of CD19 depleted cellular starting product into Cell Preparation Bag Part 2

▶ Dilution of CD19 depleted cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of CD19 depleted cellular starting product

▶ Centrifugation

Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cell product – Labeling volume
2. Resuspend the cell pellet.

▶ Incubation with the CliniMACS Anti-Biotin Reagent

1. Add 1 vial of CliniMACS Anti-Biotin Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

- **Removal of excess reagent**

1. Fill Cell Preparation Bag Part 2 with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible.
4. Resuspend the cell pellet.
5. Fill Cell Preparation Bag Part 2 with buffer.
6. Centrifuge (300×g, without brake, 15 minutes, RT).
7. Remove supernatant as much as possible.
8. Resuspend the cell pellet.
9. Adjust sample loading volume to 100 g.

6.13.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program ENRICHMENT 3.2**

See section 7.2.4 on page 138.

6.13.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.13.4 STEP 4 : CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.3 on page 192.

6.14 Enrichment of CD304 (BDCA-4) positive cells

The CliniMACS Plus CD304 (BDCA-4) System including the CliniMACS Plus Instrument, the CliniMACS CD304 (BDCA-4) Reagent, the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD304 (BDCA-4) positive cells from heterogeneous hematologic cell populations.

6.14.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 0.2×10^9 CD304 (BDCA-4) positive cells out of up to 40×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD304 (BDCA-4) Reagent	278-01	1 vial
CliniMACS Tubing Set	161-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	4×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.38: CliniMACS Materials required for enrichment of CD304 (BDCA-4) positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and three plasma transfer sets, for use during the cell preparation procedures
- **Plasma Waste Bags and Wash Waste Bags:**
  five 600 mL transfer bags suitable for centrifugation
- **Cell Collection Bag:**
  one 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set

- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of depleted cellular starting product

Add buffer: Weight of buffer to be added = 600 g – Weight of cellular starting product

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

▶ Repeat steps “Dilution of cellular starting product” and “Centrifugation” twice

1. Fill up one transfer bag to 600 g
2. Centrifuge at 300×g without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
3. Remove supernatant.

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = 95 g + Weight of empty CPB.
4. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD304 (BDCA-4) Reagent

1. Add 1 vial of CliniMACS CD304 (BDCA-4) Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 100 g.

6.14.2 STEP 2: Choice of separation program

▶ Switch-on the CliniMACS Plus Instrument

▶ Choice of separation program ENRICHMENT 3.2

See section 7.2.4 on page 138.

6.14.3 STEP 3: Installation of CliniMACS Tubing Sets

▶ CliniMACS Tubing Set and CliniMACS Tubing Set LS

See section 7.3.1 on page 150.

6.14.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.3 on page 192.

6.15 Depletion of TCRα/β positive cells

The CliniMACS Plus TCRα/β-Biotin System including the CliniMACS Plus Instrument, the CliniMACS TCRα/β-Biotin, the CliniMACS Anti-Biotin Reagent, the CliniMACS Depletion Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human TCRα/β positive cells from heterogeneous hematologic cell populations.

6.15.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 24×10^9 TCRα/β positive cells out of up to 60×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS TCRα/β-Biotin	701-48	1 vial
CliniMACS Anti-Biotin Reagent	173-01	2 vials
CliniMACS Depletion Tubing Set	261-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	Up to 4×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.39: CliniMACS Materials required for depletion of TCRα/β positive cells

Additional materials required

- **Cell Preparation Bag:**
  one 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bags and Wash Waste Bags:**
  four 600 mL (or 1,000 mL) transfer bags suitable for centrifugation
- **Cell Collection Bag:**
  One 600 mL Cell Collection Bag is already assembled to the CliniMACS Depletion Tubing Set.
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

Determine the percentage/total number of all TCRα/β-labeled cells (including unspecific TCRα/β binding, not only real TCRα/β positive cells). Consider this number for application specifications and sample parameter input (see STEP 2).

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

1. Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
2. Remove supernatant.

Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = 95 g + Weight of empty CPB
4. Resuspend the cell pellet.

Labeling of the cells: incubation with the CliniMACS TCRα/β-Biotin

1. Add 1 vial of CliniMACS TCRα/β-Biotin. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

Removal of excess conjugate: repeat this step

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible.
4. Resuspend the cell pellet and adjust the weight to 190 g after the second wash.

Magnetic labeling of the cells: incubation with the CliniMACS Anti-Biotin Reagent

1. Add 2 vials of CliniMACS Anti-Biotin Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume to 150 g. A maximum WBC concentration of 0.4×10^9 WBC/mL is recommended.

6.15.2 STEP 2: Choice of separation program

- **Switch-on the CliniMACS Plus Instrument**
- **Choice of separation program DEPLETION 3.1**
- **Sample parameter input**

See section 7.2.6 on page 145.

6.15.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Depletion Tubing Set**

See section 7.3.3 on page 170.

6.15.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.5 on page 200.

6.16 Depletion of CD45RA positive cells

The CliniMACS Plus CD45RA System including the CliniMACS Plus Instrument, the CliniMACS CD45RA Reagent, the CliniMACS Depletion Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD45RA positive cells from heterogeneous hematologic cell populations.

6.16.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

Up to 20×10^9 CD45RA positive cells out of up to 50×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalogue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS CD45RA Reagent	701-46	1 vial
CliniMACS Depletion Tubing Set	261-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.40: CliniMACS Materials required for depletion of CD45RA positive cells

Additional materials required

- **Cell Preparation Bags:**
  one 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bags and Wash Waste Bags:**
  two 600 mL transfer bags suitable for centrifugation
- **Cell Collection Bag:**
  One 600 mL Cell Collection Bag is already assembled to the CliniMACS Depletion Tubing Set.
- one pre-system filter
- locking forceps

- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes

IMPORTANT

An alternative starting cell source can be the non-target cell fraction of a CliniMACS Plus CD34 Separation. The use of the CliniMACS CD45RA Reagent is only recommended for cellular starting products containing CD45RA positive cells within the normal frequency range (up to 80%). Higher frequencies have been observed in a few donors where CD45RA is expressed by almost all PBMC due to a rare mutation within the CD45 gene. In such cases, the depletion efficiency and/or recovery may become inadequate.

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Magnetic labeling of cells

Refer to section 7.1 “STEP 1: Cell preparation and magnetic labeling” on page 119 for a detailed description of the following steps.

▶ Analysis of cellular starting product

Determine the percentage/total number of all CD45RA-labeled cells (including unspecific CD45RA binding, not only real CD45RA positive cells). Consider this number for application specifications and sample parameter input (see STEP 2).

▶ Transfer of cellular starting product into Cell Preparation Bag

▶ Dilution of depleted cellular starting product

Add buffer: Weight of buffer to be added = Weight of cellular starting product × 2

▶ Centrifugation

Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).

If the non-target cell fraction of a CliniMACS CD34 Separation is used as alternative starting cell source, the dilution step can be skipped. Continue with the centrifugation step and centrifuge the cells at 300×g (without brake) for 15 minutes at RT.

▶ Volume adjustment: labeling volume 95 g (±5 g)

1. Remove supernatant completely taking care not to resuspend the cell pellet.
2. Resuspend the cell pellet carefully after removal of supernatant.
3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: Target weight of filled CPB = 95 g + Weight of empty CPB
4. Resuspend the cell pellet.

▶ Incubation with the CliniMACS CD45RA Reagent

1. Add 1 vial of CliniMACS CD45RA Reagent. Mix contents gently.
2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.

▶ Removal of excess reagent

1. Fill Cell Preparation Bag with buffer.
2. Centrifuge (300×g, without brake, 15 minutes, RT).
3. Remove supernatant as much as possible. **Note:** Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
4. Resuspend the cell pellet.
5. Adjust sample loading volume. Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately 125 g. Cell concentration should not exceed 0.4×10^9 WBC/mL: Weight of buffer to be added (g) = 125 g – Weight of cell product after the wash (g)

6.16.2 STEP 2: Choice of separation program

▶ Switch-on the CliniMACS Plus Instrument

▶ Choice of separation program DEPLETION 3.1

▶ Sample parameter input

See section 7.2.6 on page 145.

6.16.3 STEP 3: Installation of CliniMACS Tubing Sets

▶ CliniMACS Depletion Tubing Set

See section 7.3.3 on page 170.

6.16.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.5 on page 200.

6.17 Enrichment of human IFN-gamma secreting cells with the CliniMACS Cytokine Capture System (IFN-gamma)

The CliniMACS Plus CCS (IFN-gamma) System including the CliniMACS Plus Instrument, the CliniMACS Cytokine Capture System (IFN-gamma), the CliniMACS Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human IFN-gamma secreting cells from heterogeneous hematologic cell populations.

6.17.1 STEP 1: Cell preparation and magnetic labeling

First actions required

Application capacity

IFN-gamma secreting cells out of up to 1×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells. If the application specifications are exceeded, there is the risk of insufficient specific labeling. Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Catalgue number	Quantity
CliniMACS Plus Instrument	151-01	1
CliniMACS Cytokine Capture System (IFN-gamma)	279-01	1 set
CliniMACS Tubing Set	161-01	1 tubing set
CliniMACS PBS/EDTA Buffer	700-25	3×1,000 mL bags[1]

1 Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.41: CliniMACS Materials required for enrichment of human IFN-gamma secreting cells

IMPORTANT

Store the buffer for cell preparation cold at +2 °C to +8 °C (+36 °F to +46 °F). Lower or higher ambient temperature may result in less purity and yield of the target cells.

Additional materials required

- **Cell Preparation Bags:**
  two 600 mL transfer bags suitable for centrifugation as well as two sampling site couplers and two plasma transfer sets, for use during the cell preparation procedures
- **Plasma Waste Bags and Wash Waste Bags:**
  nine 600 mL transfer bags suitable for centrifugation
- **Medium bag:**
  eight 600 mL transfer bags suitable for centrifugation
- **Cell Collection Bag (Part 2):**
  one 150 mL transfer bag suitable for centrifugation in combination with a Luer/Spike Interconnector to connect the transfer bag to the tubing set
- one 100 mL gas-permeable cell culture bag
- one pre-system filter
- locking forceps
- syringes (different sizes) and hypodermic 20-gauge needles
- human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- sample tubes
- 120 mL of AB serum or, alternatively, autologous serum, as a supplement for the media used
- 1,000 mL of cell culture medium (RPMI 1640), +4 °C (+39 °F)
- 1,100 mL of cell culture medium (RPMI 1640), +37 °C (+99 °F) supplemented with 10% (v/v) of AB serum or, alternatively, autologous serum

- 500 mL of cell culture medium (RPMI 1640, +4 °C (+39 °F) supplemented with 2% (v/v) of AB serum or, alternatively, autologous serum

▶ Equipment required

Find information regarding equipment required for a CliniMACS Plus Separation in section 4.7.

Additional equipment required for an application using the CliniMACS Plus CCS (IFN-gamma) System:

- 24 well culture dish
- MiniMACS Separation Unit
- MS Columns
- pre-separation filter (only for MS Columns)
- MACSmix Tube Rotator

▶ Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 7.1.1 on page 119.

Note: Pre-cool the centrifuge to +4 °C (+39 °F). After the secretion phase, a third Cell Preparation Bag is required to recombine the cell suspensions. Therefore, label one of the four 600 mL transfer bags (prepared with 125 mL of medium (RPMI) supplemented with 10% AB serum) as **Cell Preparation No. 3 (Separation)**.

Preparation of cellular starting product

The following sections describe the recommended in-bag procedure for the preparation of the cellular starting product using the sterile tubing connector.

- The operator must be familiar with the operation and use of the sterile tubing connector.
- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Transfer of cellular starting product into Cell Preparation Bag No. 1

1. Record the date and the start time before beginning to prepare the cellular starting product.
2. Determine the volume of the original cellular starting product by estimating 1 mL of original product as equivalent to 1 g (1 g ≙ 1 mL).
3. Holding the bag containing the cellular starting product with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the sterile tubing connector, connect the Cell Preparation Bag No. 1 to the bag containing the cellular starting product.

5. Open the locking forceps to transfer the appropriate weight of the cellular starting product (containing a maximum of 1×10^9 cells, according to the frequency of leukocytes determined before) into the Cell Preparation Bag No. 1. Visually control (by monitoring the scale) that the appropriate weight has been transferred. Close the locking forceps next to the Cell Preparation Bag.

6. Seal off the tubing and separate the bags, leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1 for further connections (see Figure 6.1). Use the heat sealer to produce three adjacent seals in the tubing. Make sure the seals are thoroughly established. Sever at the center seal. Keep the bag which contained the cellular starting product until the separation and final analysis of all cells have been accomplished.

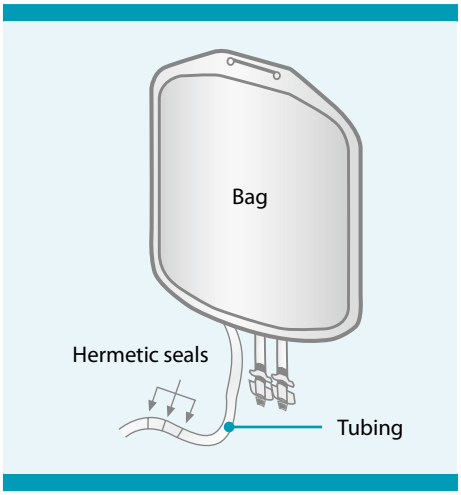


Figure 6.1: Sealing a bag

7. Tare the balance. Lay the filled Cell Preparation Bag No. 1 on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag No. 1.

8. Determine the weight of the cellular starting product by subtracting the weight of the empty Cell Preparation Bag No. 1 from the weight of the Cell Preparation Bag No. 1 filled with the original cellular starting product. Record the calculated weight.

Weight of cellular starting product (g)	=	Weight of filled Cell Preparation Bag No. 1 (g)	–	Weight of empty Cell Preparation Bag No. 1 (g)

Dilution of cellular starting product

The cellular starting product must be diluted with culture medium. Dilute the product with cold medium (+2 °C to +8 °C [+36 °F to +46 °F]) to a total volume of 500 mL. Calculate the weight of medium to be added using the following equation and record it.

Weight of medium to be added (g)	=	500 g	–	Weight of diluted cellular starting product (g)

1. Take one of the medium bags and connect it to the Cell Preparation Bag No. 1 using the sterile tubing connector.

2. Place the Cell Preparation Bag No. 1 on the balance and tare the balance. Hang the medium bag on a bag hanger.

3. Open the locking forceps next to the Cell Preparation Bag No. 1. By visually monitoring the scale on the balance, transfer the calculated weight of medium to the Cell Preparation Bag No. 1.
4. When the appropriate weight of medium has been transferred, close the locking forceps next to the Cell Preparation Bag No. 1 to stop the liquid flow.
5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1 for further connections. Disconnect the medium bag.
6. Holding the Cell Preparation Bag No. 1 with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.
7. Tare the balance and weigh the filled Cell Preparation Bag No. 1. Record the weight.
8. Determine the weight of the diluted cellular starting product by subtracting the weight of the empty Cell Preparation Bag No. 1 from the weight of the filled Cell Preparation Bag No. 1. Record the calculated weight.

Weight of diluted cellular starting product (g)	=	Weight of filled Cell Preparation Bag No. 1 (g)	–	Weight of empty Cell Preparation Bag No. 1 (g)

⚠ WARNING

Risk of contamination. If the bags are not suitable for cell preparation or not placed correctly into the centrifuge bucket, there is the risk of contamination due to bag damage. Use suitable bags, fold any loose parts of the bags or tubing downwards, taking care not to impair bag integrity and place the bags securely in the centrifuge bucket.

⚠ CAUTION

Risk of lower cell separation performance. If the bags are not placed correctly into the centrifuge bucket, there is the risk of cell loss during supernatant removal. Fold any loose parts of the bags or tubing downwards and place the bags securely in the centrifuge bucket.

Centrifugation

1. Using the sterile tubing connector, connect the empty Plasma Waste Bag No. 1 to the Cell Preparation Bag No. 1.
2. Fold any loose parts of the Cell Preparation Bag No. 1 or tubing downwards. Place the two bags securely in the centrifuge bucket.
3. Balance the loaded bucket with a suitable weighed bucket. It is essential that the centrifuge is balanced accurately.
4. Centrifuge the cells at 200×g (without brake) for 10 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).
5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag No. 1 onto the plasma extractor.

6. Open the locking forceps next to the Cell Preparation Bag No. 1. Remove the supernatant completely, taking care not to resuspend the cell pellet during removal of the supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag No. 1 to stop the liquid flow.
7. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1 for further connections. Disconnect the Plasma Waste Bag No. 1.
8. Repeat dilution and centrifugation using the second medium bag prepared with cold medium and the Plasma Waste Bag No. 2.
9. Keep the Plasma Waste Bags until the separation and final analysis of all cells have been accomplished.

Volume adjustment

1. For *in vitro* restimulation of the cells, the volume of the cell sample must be adjusted to 100 mL. When the supernatant has been removed, resuspend the pellet carefully.
2. Adjust the weight of the Cell Preparation Bag No. 1 by adding culture medium. Calculate the weight of medium to be added using the following equation:

Weight of filled Cell Preparation Bag No. 1 after volume adjustment (g)	=	100 g	+	Weight of empty Cell Preparation Bag No. 1 (g)

3. Using the sterile tubing connector, connect the Cell Preparation Bag No. 1 to the prepared Medium Bag 10% (containing 600 mL pre-warmed culture medium supplemented with 10% AB serum). Hang the Medium Bag 10% on a bag hanger.
4. Tare the balance. Place the Cell Preparation Bag No. 1 with the cell pellet on the balance.
5. Open the locking forceps next to the Cell Preparation Bag No. 1. Fill the Cell Preparation Bag No. 1 with medium until the calculated "Weight of filled Cell Preparation Bag No. 1 after volume adjustment" is reached. Close the locking forceps next to the Cell Preparation Bag No. 1 to stop the liquid flow.
6. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Medium Bag 10%.
7. Using the sterile tubing connector, connect the filled Cell Preparation Bag No. 1 to a 100 mL gas-permeable culture bag. Open the locking forceps next to the Cell Preparation Bag No. 1 and completely transfer the content.

⚠ CAUTION

Risk of lower performance. If the bags are not suitable for cell preparation, there is the risk of contamination due to bag damage. Use gas-permeable culture bags suitable for cell preparation.

Labeling of cells

One CliniMACS Cytokine Capture System (IFN-gamma), consisting of two vials (7.5 mL each), is ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Target sample and control sample

The protocol describes the labeling procedure of the "target sample" and the "control sample". Both samples are processed in parallel, the control sample being treated exactly the same as the target sample, but without addition of antigen. Therefore, the analysis of the control sample gives information on spontaneous cytokine secretion.

In vitro restimulation

IMPORTANT

- *A small negative control sample (control sample), treated exactly the same as the antigen-stimulated target sample but without addition of antigen, should be included as a measure of spontaneous cytokine secretion.*
- *For in vitro restimulation the optimal cell density is 0.5–1×10^9 cells/cm^2 and 1×10^9 cells/mL.*

1. Using a sterile syringe (1 mL) with a 20-gauge needle, take a small aliquot (e.g., 1 mL = appr. 1×10^9 cells) of the cells to serve as **control sample (unstimulated)**.
2. Culture the aliquot under optimal conditions in one well of a 24 well culture dish.
3. Inject the antigen to the cells in the gas-permeable cell culture bag **(target sample)** at predetermined concentration via one of the ports using a syringe.
4. Incubate both samples at +37 °C (+99 °F) and 5–7.5% CO^2 for an appropriate time in the incubator. When proteins or cell lysates are used as antigens, the incubation time is between 4 and 16 hours. For peptides the incubation time is 3 to 6 hours. As the optimal stimulation time is influenced by specific characteristics of the antigen, Miltenyi Biotec recommends to perform dedicated validation procedures in order to gain best result.

Labeling with the CliniMACS IFN-gamma Catchmatrix Reagent

Record the lot number and use-by date of the CliniMACS IFN-gamma Catchmatrix Reagent.

Target sample

IMPORTANT

For the secretion phase, the vessel of choice must be of sufficient size to allow the addition of at least 1 volume of cold buffer at the end of the secretion phase. The cell density must not exceed 1×10^6 cells/mL. Therefore, 1×10^9 cells must be incubated for secretion in a total of 1,000 mL of warm culture medium.

1. Use the sterile tubing connector to connect the culture bag to the Cell Preparation Bag No. 2 (Secretion) and transfer the cells. Ensure the complete transfer of the cells into the Cell Preparation Bag No. 2. Close the locking forceps next to the Cell Preparation Bag No. 2 to stop the liquid flow.
2. Use the heat sealer to seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 2 for further connections. Disconnect the empty culture bag.
3. Use the sterile tubing connector to connect the prepared Medium Bag 2% (containing 500 mL cold (+2 to +8 °C [+36 °F to +46 °F]) culture medium supplemented with 2% AB serum) to the Cell Preparation Bag No. 2.
4. Open the locking forceps next to the Cell Preparation Bag No. 2. Fill the Cell Preparation Bag No. 2 up to 600 mL. When the appropriate weight of medium has been transferred, close the locking forceps next to the Cell Preparation Bag No. 2 to stop the liquid flow.
5. Use the heat sealer to seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 2 for further connections. Disconnect the Medium Bag 2%.
6. Use the heat sealer to connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag No. 2.
7. Fold any loose parts of the bag or tubing downwards. Place the two bags securely in the centrifuge bucket.
8. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.
9. Centrifuge cells at 300×g (without brake) for 10 minutes at +4 °C to +8 °C (+39 °F to +46 °F).
10. Place the Cell Preparation Bag No. 2 on the plasma extractor. Open the locking forceps next to the Cell Preparation Bag No. 2. Remove the supernatant completely, taking care not to disturb the cell pellet. Close the locking forceps next to the Cell Preparation Bag No. 2 to stop the liquid flow.
11. Use the heat sealer to seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 2 for further connections. Disconnect the Wash Waste Bag No. 1.
12. Resuspend the cell pellet in the residual volume after removal of the supernatant.

13. Attach a sampling site coupler to the Cell Preparation Bag No. 2 and disinfect the septum. Use an appropriate sterile syringe and needle to remove 7.425 mL from the CliniMACS IFN-gamma Catchmatrix Reagent vial. A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20-gauge needle.
14. Holding the Cell Preparation Bag No. 2 with both hands, mix the contents thoroughly by using a gentle rotating motion.
15. Using the injection port on the sampling site coupler, inject the entire volume of the reagent into the Cell Preparation Bag No. 2. Take care not to puncture the bag.
16. Resuspend the cells and incubate them for 5 minutes on ice. Record the incubation time.

Control sample (unstimulated)

1. Transfer the cells to a 15 mL closable tube.
2. Add cold (+2 °C to +8 °C [+36 °F to +46 °F]) culture medium supplemented with 2% AB serum up to 15 mL.
3. Centrifuge cells at 300×g (without brake) for 10 minutes at +4 °C to +8 °C (+39 °F to +46 °F) and carefully remove supernatant completely.
4. Resuspend cell pellet in 100 µL of cold (+2 °C to +8 °C [+36 °F to +46 °F]) culture medium supplemented with 2% AB serum.
5. Add the remaining 75 µL of the CliniMACS IFN-gamma Catchmatrix Reagent and incubate the suspension for 5 minutes on ice.

Secretion phase

Target sample

1. Take the four pre-filled transfer bags (including the labeled Cell Preparation Bag No. 3) containing 125 mL of warm (+37 °C [+99 °F]) culture medium supplemented with 10% AB serum.
2. Using the sterile tubing connector, connect the Cell Preparation Bag No. 2 to the prepared Medium Bag 10% (containing pre-warmed (+37 °C [+99 °F]) culture medium supplemented with 10% AB serum).
3. Add 500 mL of the pre-warmed culture medium to the cell sample in the Cell Preparation Bag No. 2. Immediately start measuring the 45 minutes incubation time.
4. Distribute 125 mL of the resulting cell suspension in each of the four transfer bags by serial attachment of the transfer bags to the Cell Preparation Bag No. 2 using the sterile tubing connector. By visually monitoring the scale on the balance, transfer the appropriate weight of cell suspension. This procedure results in a total of 250 g of cell suspension in each of the four transfer bags. After transfer, seal off the tubing to disconnect the transfer bags using the heat sealer.

5. Incubate the cells at +37 °C (+99 °F) for the remainder of the 45 minutes with continuous slow rotation (max 50 rpm) until the end of incubation time. Record the incubation time.

6. Take a plasma transfer set and ensure that the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

7. After the 45 minutes, connect the buffer bag to the Cell Preparation Bag No. 3 using the sterile tubing connector. Add cold (+2 °C to +8 °C [+36 °F to +46 °F]) buffer to each of the four transfer bags (at least one volume, i.e., 250 mL each) by serial attachment of the transfer bags to the buffer bag. Allow the cells to cool down by placing the bags on ice for 10 minutes.

8. Using the sterile tubing connector connect one Wash Waste Bag (Wash Waste Bags Nos. 2 to 5) to each of the four transfer bags.

9. Centrifuge cells at 300×g (without brake) for 10 minutes at +4 °C (+39 °F). Using the plasma extractor, completely remove supernatant of all bags.

10. Using the heat sealer, seal off the tubing of each bag. Disconnect the Wash Waste Bags.

11. Take a plasma transfer set and ensure that the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of a buffer bag.

12. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag No. 3.

13. Slide the clamp on the plasma transfer set to the open position and fill the Cell Preparation Bag No. 3 with 200 mL of buffer.

14. When the appropriate weight of buffer is transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 3. Disconnect the buffer bag.

16. Using the sterile tubing connector, attach one of the other three transfer bags containing the cells to the Cell Preparation Bag No. 3 and recombine both suspensions.

    IMPORTANT
    The divided samples have to be recombined in the Cell Preparation Bag No. 3 before centrifugation.

17. Repeat this procedure until the cell suspensions from all four transfer bags are recombined in the Cell Preparation Bag No. 3.

18. After the cells have been recombined, connect the buffer bag to the Cell Preparation Bag No. 3 using the sterile tubing connector.

19. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer up to 500 mL.

20. When the appropriate weight of buffer is transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag No. 3.

21. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag No. 3 for further connections. Disconnect the buffer bag.
22. Using the sterile tubing connector, connect the empty Wash Waste Bag No. 6 to the Cell Preparation Bag No. 3.
23. Fold any loose parts of the Cell Preparation Bag No. 3 or tubing downwards. Place the two bags securely in the centrifuge bucket.
24. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.
25. Centrifuge cells at 300×g (without brake) for 10 minutes at +4 °C (+39 °F).

Control sample (unstimulated)

1. Add warm (+37 °C [+99 °F °F]) culture medium supplemented with 10% AB serum to dilute the sample to a maximum of 1×10^6 cells/mL, i.e., a total volume of 10 mL must be used for the 1×10^9 cells.
2. Incubate the cells in a closed tube for 45 minutes at +37 °C (+99 °F) under slow continuous rotation using the MACSmix Tube Rotator (max. 50 rpm).
3. After 45 minutes, fill up the tube with cold (+4 °C [+39 °F]) buffer and place the tube on ice for 5–10 minutes.
4. Centrifuge cells at 300×g (without brake) for 10 minutes at +4 °C to +8 °C (+39 °F to +46 °F).

Magnetic labeling of cells with the CliniMACS IFN-gamma Enrichment Reagent

Record the lot number and use-by date of the CliniMACS IFN-gamma Enrichment Reagent.

Target sample

1. After the centrifugation step carefully remove supernatant, using the plasma extractor, as described above and resuspend in residual volume. Close the locking forceps next to the Cell Preparation Bag No. 3 after removal.
2. Use the heat sealer to seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 3 for further connections. Disconnect the Wash Waste Bag No. 6.
3. Attach a sampling site coupler to the Cell Preparation Bag No. 3 and disinfect the septum. Use an appropriate sterile syringe and needle to remove 7.425 mL from the CliniMACS IFN-gamma Enrichment Reagent vial. A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20-gauge needle.

4. Using the injection port on the sampling site coupler, inject the reagent into the Cell Preparation Bag No. 3. Take care not to puncture the bag. Immediately start counting the incubation time of 15 minutes. Record the incubation start time.
5. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
6. Incubate the cells for 15 minutes on ice. Record the incubation stop time.
7. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag No. 3.
8. Open the locking forceps next to the Cell Preparation Bag No. 3. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag No. 3 up to 500 mL with cold (+2 °C to +8 °C [+36 °F to +46 °F]) buffer.
9. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position. Close the locking forceps next to the Cell Preparation Bag No. 3. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.
10. Using the sterile tubing connector, connect the empty Wash Waste Bag No. 7 to the Cell Preparation Bag No. 3.
11. Fold any loose parts of the bag or tubing downwards. Place the two bags securely in the centrifuge bucket.
12. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.
13. Centrifuge at 300×g (without brake) for 10 minutes at +4 °C (+39 °F).
14. Carefully remove the supernatant as described above and resuspend the cells in 100 mL of cold (+2 °C to +8 °C [+36 °F to +46 °F]) buffer.
15. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Take care not to puncture the bag. Transfer the sample into a sample tube. Label the tube as ORIGINAL TARGET SAMPLE (should include patient identification).

    IMPORTANT

    Ensure that the cells are mixed thoroughly before taking the sample. In order to get a representative sample, use a syringe with a capacity of at least 5 mL.
16. Retain the cell sample for cell analysis stored in the cold (+2 °C to +8 °C [+36 °F to +46 °F]).

Control sample (unstimulated)

1. After the centrifugation carefully remove supernatant.
2. Resuspend the cells in 100 µL of cold buffer (+2 °C to +8 °C [+36 °F to +46 °F]).
3. Add 75 µL of the remaining CliniMACS IFN-gamma Enrichment Reagent.
4. Incubate the cells for 15 minutes on ice.

5. Fill the vessel with cold buffer (+2 °C to +8 °C [+36 °F to +46 °F]) and centrifuge at 300×g (without brake) for 10 minutes at +4 °C (+39 °F).
6. Carefully remove the supernatant and resuspend the cells in 1 mL of cold (+2 °C to +8 °C [+36 °F to +46 °F]) buffer.
7. Remove a small sample (appr. 100 µL) of the cell suspension and transfer it into a tube, labeled as ORIGINAL CONTROL SAMPLE fraction before separation. Store in the cold until analysis.

Magnetic separation

Target sample

The magnetic separation of the target sample is performed using the CliniMACS Plus Instrument. After the separation procedure, process the control sample as described below.

Control sample (unstimulated)

1. Prepare two MS Columns by rinsing them with 500 µL of cold (+2 °C to +8 °C [+36 °F to +46 °F]) buffer and discard the effluent.
2. Place the first column into the magnetic field of the MiniMACS Separator.
3. Pass the cells through a pre-separation filter to remove clumps.
4. Apply the cell suspension onto the column.
5. Collect the total effluent (containing the non-labeled cell fraction). Wash the column by adding 500 µL of cold buffer onto the column. Perform a total of three washing steps by successively applying 500 µL of buffer onto the column once the column reservoir is empty.
6. Remove the column from the separator, place the second column into the separator, and put the first column on top of the second one.
7. Pipette 1 mL of cold buffer onto the first column. Immediately flush out the fraction with the magnetically labeled cells by firmly applying the plunger, supplied with the column, directly onto the second column.
8. Collect the non-labeled cells that pass through and wash the column with 500 µL of cold buffer. Perform a total of three washing steps by successively applying 500 µL of buffer onto the column, once the column reservoir is empty.
9. Remove the second column from the separator and place the column on a suitable collection tube labeled as positive fraction.
10. Pipette 0.5 mL of cold buffer onto the column. Immediately flush out the fraction with the magnetically labeled cells by firmly applying the plunger, supplied with the column.
11. Keep the target cell fraction in the cold until analysis.

6.17.2 STEP 2: Choice of separation program

- **Choice of separation program ENRICHMENT 3.2**

See section 7.2.4 on page 138.

6.17.3 STEP 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 7.3.1 on page 150.

6.17.4 STEP 4: CliniMACS Plus Separation

- **Separation procedure**
- **Disconnect bags and record process code**
- **Unload tubing set and shutdown**
- **Analysis of cells**

See section 7.4.3 on page 192.

7 Detailed explanation of STEP 1–4

7.1 STEP 1: Cell preparation and magnetic labeling

This chapter gives the detailed explanations of the procedures that are needed for cell separation using the CliniMACS Plus System.

7.1.1 First actions required

For the materials required for a particular application, refer to the application-specific section (see section 5.2 on page 37).

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Preparation of bags

Refer to the application-specific section for the bags needed:

- **Cell Preparation Bag:** Insert a sampling site coupler (or equivalent) into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

  IMPORTANT
  Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

- **Cell Collection Bag:**

  - CliniMACS Tubing Set/CliniMACS Tubing Set LS: Insert a Luer/Spike Interconnector (or equivalent) into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

  - CliniMACS Depletion Tubing Set: One Cell Collection Bag is already attached to the tubing set. Insert a Luer/Spike Interconnector (or equivalent) into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

7.1.2 Magnetic labeling of cells

The following sections describe the recommended in-bag procedure for the preparation of the cell product using the sterile tubing connector.

- The operator must be familiar with the operation and use of the sterile tubing connector.
- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

IMPORTANT

- *All bag handling should be done in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the sterile tubing connector may be performed outside the laminar flow hood.*
- *Perform sample preparation and cell separation at the temperature conditions defined in the application-specific section. Lower or higher ambient temperature will result in less purity and yield of the target cells.*

In short, the magnetic labeling of the cell product is performed by the following steps:

1. analysis of cellular starting product
2. transfer of cellular starting product into Cell Preparation Bag
3. dilution of the cellular starting product
4. centrifugation
5. for the CliniMACS Plus Anti-Biotin System and CliniMACS Plus TCRα/β-Biotin System only: labeling of the cells (incubation with the primary antibody/CliniMACS TCRα/β-Biotin) and removal of excess conjugate (1st wash)
6. volume adjustment
7. for the CliniMACS Plus CD3 and CD3/CD19 Systems only: incubation with clinical grade immunoglobulin
8. magnetic labeling of the cells (incubation with CliniMACS Reagent)
9. removal of excess reagent
10. volume adjustment for cell separation

Analysis of cellular starting product

Before starting the preparation of the cellular starting product, determine the following parameters:

- total number of leukocytes
- percentage of cells to be labeled
- total number of leukocytes to be labeled
- viability

Ensure working in within the application specification.

Recheck the parameters after the labeling procedure. This is necessary for the staged-loading programs (ENRICHMENT 1.1/DEPLETION 2.1/DEPLETION 3.1), as the following three sample parameters have to be entered in the software during the setup of the CliniMACS Plus Instrument prior to loading the tubing set onto the instrument:

- concentration of leukocytes per mL
- percentage of cells to be labeled
- final volume of the cell sample (sample loading volume)

Other tests might be required depending on the intended use of the cells (e.g., number of T cells, phenotype). Record all data.

Transfer of cellular starting product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the cellular starting product.
2. Determine the volume of the cellular starting product by assuming 1 mL of cellular starting product as equivalent to 1 g (1 g ≙ 1 mL).
3. Holding the cellular starting product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
4. Using the sterile tubing connector, connect the Cell Preparation Bag to the original cellular starting product bag.
5. Open the locking forceps to transfer the cellular starting product into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps on the tubing as close to the bag as possible.

6. Seal off the tubing and separate bags, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see Figure 7.1). Use the heat sealer to produce three adjacent seals in the tubing. Make sure the seals are thoroughly established. Sever at the center seal. Keep the original leukapheresis/cellular starting bag until the separation and final analysis of all cells have been accomplished.

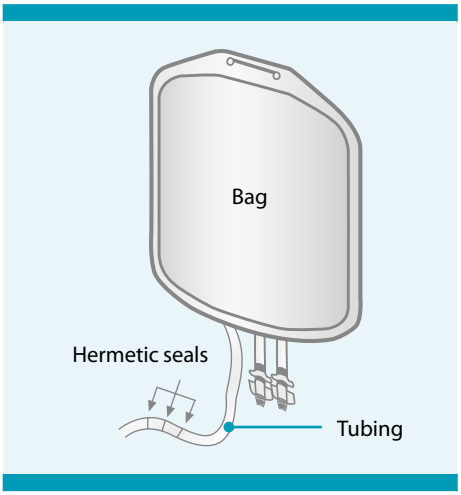


Figure 7.1: Sealing a bag.

7. For taking a sample, disinfect the septum of the sampling site coupler. Mix the contents of the bag. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS/CELLULAR STARTING PRODUCT (This should include patient identification) and retain for cell analysis.

   IMPORTANT
   Ensure that the cellular starting product is mixed thoroughly before taking the sample. In order to get a representative sample, use a syringe with a capacity of at least 5 mL.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight.

9. Determine the weight of the cellular starting product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with cellular starting product. Record the calculated weight.

Weight of cellular starting product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Dilution of cellular starting product

Refer to the application-specific section for the dilution ratio needed.

The cellular starting product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the weight of buffer to be added using either of the following equations (1 or 2) depending on the application and record it.

(1)	Weight of buffer to be added (g)	=	600 g	–	Weight of cellular starting product (g)
(2)	Weight of buffer to be added (g)	=	Weight of cellular starting product (g)	×	2

1. Take a plasma transfer set and make sure that the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.
2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
3. Place the Cell Preparation Bag on the balance and tare the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.
5. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps on the tubing as near to the bag as possible.
6. Use the heat sealer to seal off the tubing between the clamp and the locking forceps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.
8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
9. Determine the weight of the diluted cellular starting product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of diluted cellular starting product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

⚠ WARNING

Risk of contamination. If the bags are not suitable for cell preparation or not placed correctly into the centrifuge bucket, there is the risk of contamination due to bag damage. Use suitable bags, fold any loose parts of the bags or tubing downwards, taking care not to impair bag integrity and place the bags securely in the centrifuge bucket.

⚠ CAUTION

Risk of lower cell separation performance. If the bags are not placed correctly into the centrifuge bucket, there is the risk of cell loss during supernatant removal. Fold any loose parts of the bags or tubing downwards and place the bags securely in the centrifuge bucket.

1. Using the sterile tubing connector, connect the empty Plasma Waste Bag to the Cell Preparation Bag.
2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.
4. Centrifuge the cells at conditions defined in the application-specific section. Record the centrifugation conditions.
5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor and the connected Waste Bag on the table top balance.

Volume adjustment

For magnetic labeling of the cells, the optimal weight of the cell product varies depending on the application. Refer to the labeling volume in the application-specific section.

Depending on the application, it may be necessary either to only remove some amount of the supernatant to reach the labeling volume (tailored supernatant removal) or remove the supernatant completely and add buffer to reach the labeling volume (complete supernatant removal). Refer to the application-specific section for the applicable option and continue accordingly.

IMPORTANT

- *Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly.*
- *During removal of supernatant be careful not to lose cells.*

Tailored supernatant removal

Calculate the weight of supernatant to be removed to adjust the sample to the labeling volume using the following equation:

Weight of supernatant to be removed (g)	=	Weight of cell product after the wash (g)	–	Labeling volume (g)

Record the weight of supernatant to be removed.

1. Place the empty Plasma Waste Bag or Wash Waste Bag No. 1 on the balance and tare the balance.
2. Open the locking forceps next to the Cell Preparation Bag. By visually monitoring the scale on the balance, remove the supernatant using the plasma extractor. Carefully press out excess supernatant until the calculated "weight of supernatant to be removed" is reached.
3. When the appropriate weight of supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed.

4. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag or Wash Waste Bag No. 1.
5. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
6. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
7. Determine the weight of the cell product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of cell product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

8. Keep the Plasma Waste Bag or Wash Waste Bag until the final separation and analysis of the all cells have been accomplished.

Complete supernatant removal

Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted cell product using the following equation:

Target weight of CPB filled with washed cell product (g)	=	Labeling volume (g)	+	Weight of empty CPB (g)

1. Insert the spike of a plasma transfer set to a port of a buffer bag. Confirm the clamp on the plasma transfer set is in the closed position.
2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
3. Tare the balance. Place the Cell Preparation Bag with the cell pellet on the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer until the calculated “Target weight of Cell Preparation Bag filled with washed cell product” is reached. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.
5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
8. Determine the weight of the cell product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of cell product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been accomplished.

Labeling of the cells (for the CliniMACS Plus Anti-Biotin System and CliniMACS Plus TCRα/β-Biotin System only)

Incubation with the CliniMACS TCRα/β-Biotin

The CliniMACS TCRα/β-Biotin vial is ready to use and sufficient for the application as described below. The conjugate is not for parenteral administration.

Store the conjugate at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The conjugate must be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the conjugate is printed on the vial label. DO NOT use the conjugate after the use-by date.

1. Record the lot number and use-by date of the CliniMACS TCRα/β-Biotin.
2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one vial (7.5 mL). A 10 mL syringe is sufficient to remove the content of one vial. The syringe should be equipped with a 20 gauge needle.
3. Using the injection port on the sampling site coupler, inject the entire volume of conjugate into the Cell Preparation Bag (optionally, transfer the content of the Cell Preparation Bag into a new transfer bag to ensure high depletion efficiency). Immediately start the incubation period of 30 minutes.
4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.
5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm, and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record the incubation stop time.

Incubation with the primary antibody (biotinylated antibody of another manufacturer)

1. Resuspend cell pellet and label cells with primary biotinylated antibody at time and titer recommended by the manufacturer of the antibody. Ensure that all cells are resuspended. (The primary antibody should be used at its optimal titer, i.e., with optimal staining intensity and no background staining. Follow the instructions for use, provided by the manufacturer of the primary antibody.)
2. Incubate the Cell Preparation Bag on an orbital shaker at 25 rpm. Follow the instructions for use, provided by the manufacturer of the primary antibody.

Removal of excess conjugate (1st wash) (for the CliniMACS Plus Anti-Biotin System and CliniMACS Plus TCRα/β-Biotin System only)

Dilute the cell product with buffer to a total weight of 600 g. Calculate the weight of buffer to be added using the following equation and record it.

Weight of buffer to be added (g) = 600 g – Weight of labeled cell product (g)

1. Dilute the cell product following the steps described in the step "Dilution of cellular starting product" (steps 1–8) to a total weight of 600 g.
2. Determine the weight of cell product after the removal of excess conjugate (1st wash) wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

   Weight of cell product after removal of excess conjugate (1st wash) (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)
3. Using the sterile tubing connector, connect the empty Wash Waste Bag to the Cell Preparation Bag.
4. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.
5. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.
6. Centrifuge the cells under conditions defined in the application-specific section.
7. Remove the bags from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor and the connected Waste Bag on the table top balance.
8. For the required number of washes, see the application-specific section.
9. Adjust the weight of the cell suspension as described in the section "Volume adjustment" to approximately 190 g, for magnetic labeling of the cells with the CliniMACS Anti-Biotin Reagent.

Incubation with clinical grade immunoglobulin (for the CliniMACS Plus CD3 or CD3/CD19 Systems only)

IMPORTANT

Note that human IgG is not a component of the CliniMACS System. Use only pharmaceutical grade human IgG approved in your country. Carefully read the package insert of the human IgG used; in particular the section regarding hypersensitivity reactions and the risk of infection that human IgG as a blood derived product brings to all patients. All risks arising from these materials must be evaluated by the user.

Disinfect the septum of the sampling site coupler of the Cell Preparation Bag. Use an appropriate sterile syringe and needle to add 1.5 mg human IgG per mL cell product (e.g., 1.6 mL of Gamunex 10% for 95 mL cell product). Mix the contents thoroughly. Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion (e.g., 25 rpm on orbital rotator).

Magnetic labeling of the cells: incubation with the CliniMACS Reagent

The CliniMACS Reagent vials are ready to use and sufficient for the application as described below. The reagents are not for parenteral administration.

Store the reagents at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The reagents must be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the reagents are printed on the vial label. DO NOT use the reagents after the use-by date.

1. Record the lot number and use-by date of the CliniMACS Reagent(s).
2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one vial or b) two reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial, or respectively, a 20 mL syringe is sufficient to remove the contents of two reagent vials. The syringe should be equipped with a 20-gauge needle.
3. Using the injection port of the sampling site coupler, inject the entire volume of the reagent into the Cell Preparation Bag (optionally, in depletion applications transfer the content of the Cell Preparation Bag into a new transfer bag to ensure high depletion efficiency). The incubation time starts at this point.
4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm, and ensure that the bag is not creased or bent. Incubate the bag in the conditions and duration defined in the application-specific section.

Removal of excess reagent

To remove the unbound reagent after incubation is completed, washing the cells is required. Refer to the application-specific section for the required number of washes.

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is in the closed position.
2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
3. Place the Cell Preparation Bag on the balance and tare the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer to 600 mL. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.
5. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
6. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly with a gentle rotating motion.
7. Using the sterile tubing connector, connect the empty Wash Waste Bag to the Cell Preparation Bag.
8. Fold any loose parts of the bags or tubing downwards. Transfer the Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.
9. Centrifuge the cells in the conditions and duration defined in the application-specific section.
10. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.
11. Carefully hang the Cell Preparation Bag on the plasma extractor.
12. Place the Wash Waste Bag on the balance and tare the balance.
13. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow.
14. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag.
15. Keep the Wash Waste Bag until the separation and final analysis of all cells have been accomplished.
16. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
17. If more washes are required (e.g., for enrichment of CD34 positive cells), repeat steps 1–16.

18. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

19. Determine the weight of the cell product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of cell product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

20. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to the appropriate sample loading volume according to the application using the following equation. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

Weight of buffer to be added (g)	=	Sample loading volume (according to application)	–	Weight of cell product after the wash (g)

21. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

22. Place the Cell Preparation Bag on the balance and tare the balance.

23. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

24. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

25. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

26. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

27. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for analysis.

28. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

29. Determine the weight of the cell product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of cell product after addition of buffer (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

▶ Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

7.2 STEP 2: Choice of separation program

7.2.1 Switch-on the CliniMACS Plus Instrument

Switch on the CliniMACS Plus Instrument by using the ON/OFF switch located on the rear panel of the instrument. Record the start time of the instrument run.

The window indicates the main screen in section "Installation" of the CliniMACS Plus Instrument User Manual.

To proceed to the program menu, press **ENT**.

7.2.2 CD34 SELECTION 1/2

IMPORTANT

- *CD34 SELECTION 1 must only be used in combination with the CliniMACS Tubing Set (REF 161-01), while CD34 SELECTION 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Carefully check the tubing set prior to installation.*
- *Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step. To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

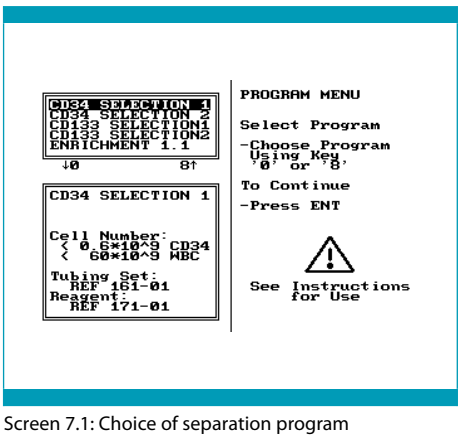
Choice of separation program CD34 SELECTION 1/2

The window indicates Screen 7.1.

Depending on the application choose CD34 SELECTION 1 or CD34 SELECTION 2.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys **0** and **8**.

To proceed with the highlighted program, press **ENT**.



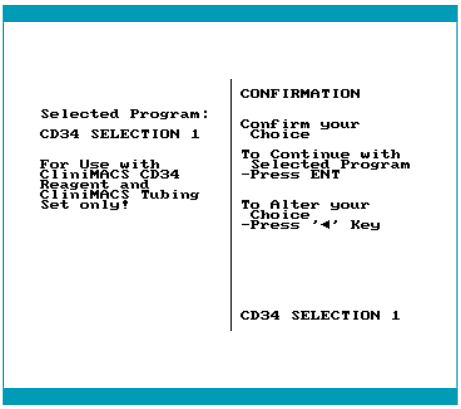
Screen 7.1: Choice of separation program

Confirmation

The window indicates Screen 7.2.

To confirm the separation program and proceed, press ENT.

If not, press ◄ to return to the previous step in order to amend the choice.



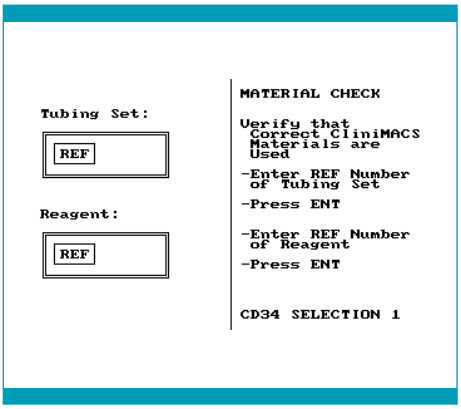
Screen 7.2: Confirmation

Material check

The window indicates Screen 7.3.

CD34 SELECTION 1 and CD34 SELECTION 2 are optimized for the enrichment of CD34 positive cells.

To confirm the suitable tubing set is available and the proper reagent has been used for cell labeling, enter the respective catalogue number (REF) in the query box. The instrument software will check whether the materials can be used in combination with the chosen program.



Screen 7.3: Material check

1. Enter catalogue number of the tubing set to be used for automated cell separation. To confirm and proceed, press ENT.
2. Enter catalogue number of the reagent that has been used for cell labeling. To confirm and proceed, press ENT.

If the catalogue number of a tubing set or a reagent not specified for the chosen separation program has been entered, a message appears. Press ENT to confirm and enter the correct catalogue number again. If the number entered is still incorrect, the message will appear a second time. After pressing ENT the program will return to the program menu (see Screen 7.1).

IMPORTANT

- *CD34 SELECTION 1 must only be used in combination with the CliniMACS Tubing Set (REF 161-01), while CD34 SELECTION 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Carefully check the tubing set prior to installation.*
- *To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

If the material check has been successful, the program continues automatically with the instructions to install the tubing set.

▶ **Proceed to STEP 3.**

7.2.3 ENRICHMENT 1.1

IMPORTANT

- *The separation program ENRICHMENT 1.1 must only be used with the CliniMACS Tubing Set (REF 161-01).*
- *Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step. To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

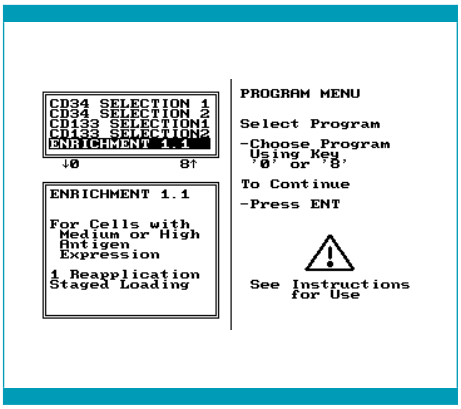
Choice of separation program ENRICHMENT 1.1

The window indicates Screen 7.4.

Choose ENRICHMENT 1.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys **0** and **8**.

To proceed with the highlighted program, press **ENT**.



Screen 7.4: Choice of separation program

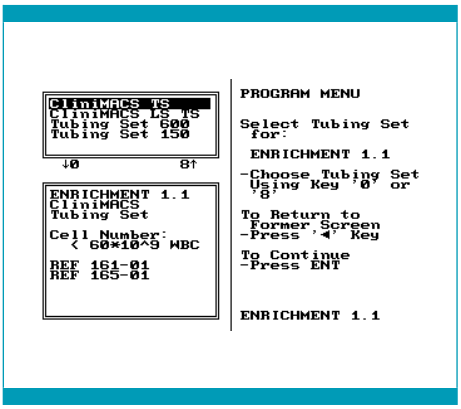
Selection of the tubing set

The window indicates Screen 7.5.

Confirm that the correct program has been chosen. If not, press ◀ to return to the previous step in order to amend the choice.

Select the tubing set by highlighting it in the box at the top left-hand side with the black bar. Move the bar up and down by using the keys 0 and 8.

The window below indicates the relevant data like capacity and catalogue number of the selected tubing set.



Screen 7.5: Selection of the tubing set

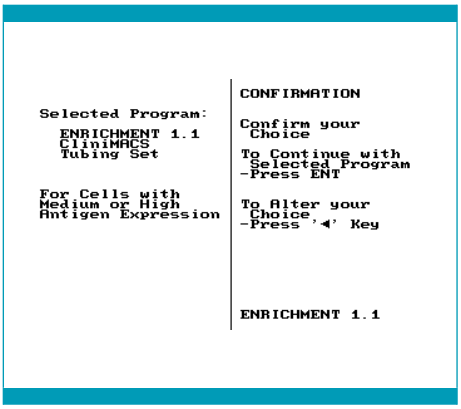
To continue with the selected combination of separation program and tubing set, press ENT.

Confirmation

The window indicates Screen 7.6.

To confirm the combination of separation program and tubing set and to proceed, press ENT.

If not, press ◀ to return to the previous step in order to amend the choice.



Screen 7.6: Confirmation

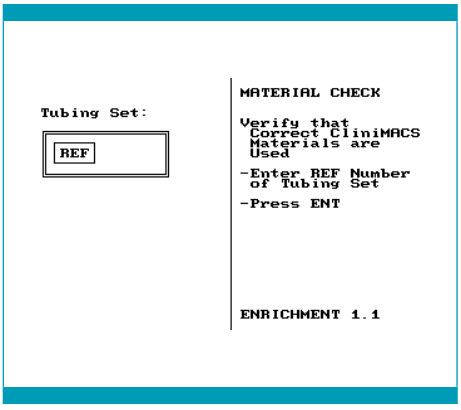
Material check

The window indicates Screen 7.7.

The query of the catalogue number (REF) of the tubing set serves as a security check to ensure that the selected tubing set can be used with the program selected.

Enter catalogue number of the selected tubing set and press ENT to proceed.

If a non-corresponding catalogue number has been entered, a message appears. To confirm press ENT and enter the correct catalogue number. If the entered number is still wrong, the message appears a second time. After pressing ENT the program will return to the program menu (see Screen 7.4).

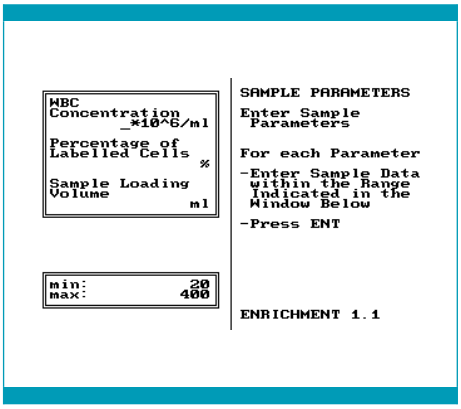


Screen 7.7: Material check

Sample parameter input

If the material check has been successful, the program continues automatically with the query for the sample parameters that are necessary to adjust the separation to each individual sample and to provide the operator with important information for required buffer and bag volumes.

The window indicates Screen 7.8.



Screen 7.8: Sample parameter input

1. Start with entering the WBC concentration of the sample. The allowed range is shown in the box on the bottom left-hand side. Press ◄ to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal. To continue with the next input, press ENT.

   IMPORTANT
   All sample parameters required refer to the sample post cell labeling and washing procedure. The allowed range shown in the box on the bottom left-hand side always takes into account the previous input. After each input the software calculates and adjusts the accepted range of the next query.

   ⚠ CAUTION

   Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss. Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, this will result in increased processing time and enlarged volume of the target cell fraction. Always ensure to enter the correct parameters.

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”). To continue with the next input, press ENT.

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”).

To continue, press ENT.

Calculation

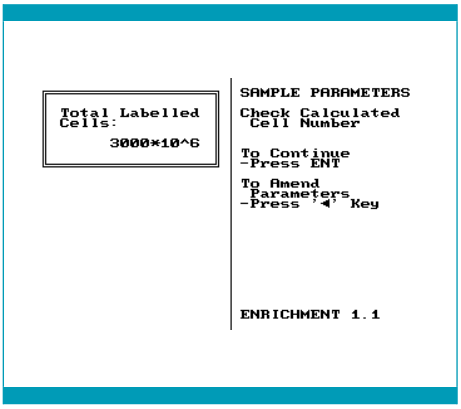
The CliniMACS Plus Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens:

- The result of the calculation is shown on a screen similar to Screen 7.9. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT.

  A correction of the data is possible. Press ◄ to return to the previous screen for correction.

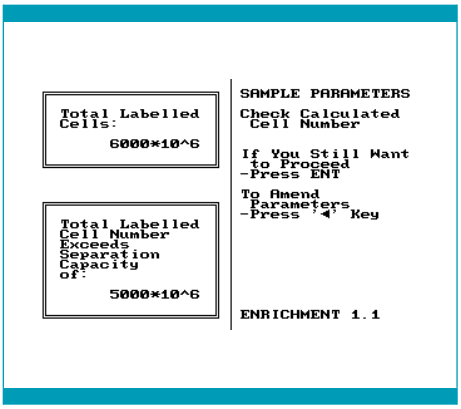


Screen 7.9: Calculation

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS Plus System a screen similar to Screen 7.10 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press ◄ to return to the previous Screen 7.8 and to correct the sample parameters.

  To avoid overloading the system, split the sample and determine the volume of each portion. Press ◄ to return to the previous Screen 7.8. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To continue, press ENT.



Screen 7.10: Calculation: Cell number exceeds separation capacity

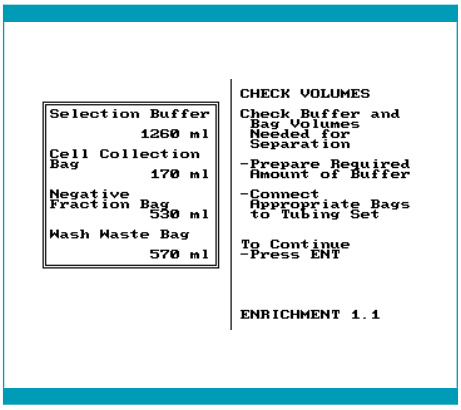
Volume information

From the total number of labeled cells (see section “Calculation”) the software calculates the number of separation stages, the amount of buffer needed for the entire separation and the liquid volumes that will be collected in Negative Fraction Bag, Buffer Waste Bag and Cell Collection Bag.

Standard values are the following:

- CliniMACS PBS/EDTA Buffer: 1,000 mL
- Negative Fraction Bag: 500 mL
- Buffer Waste Bag: 500 mL
- Cell Collection Bag: 150 mL

If one of the calculated values exceeds these standard values, Screen 7.11 appears to inform about the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set (capacity approximately 500 mL) by alternative bags of appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.



Screen 7.11: Check volumes

1. Confirm the requested amount of buffer is available. Do not attach more than three liters of buffer on the bag hanger.

   NOTICE

   Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 3 kg. Do not overload the bag hangers.

2. Open the tray of the tubing set under sterile conditions and replace the original bags with larger ones if necessary. Ensure unrestricted flow to these bags.
3. Attach a Cell Collection Bag of sufficient size to the free luer connector. If not specified on Screen 7.11, the maximum elution volume of the target cell fraction is 150 mL. Ensure that unrestricted flow to the Cell Collection Bag is possible.

IMPORTANT

- *Ensure the required amount of buffer is available and the volume of the bags mentioned on Screen 7.11 is sufficient. Otherwise the performance of the separation will be compromised.*
- *Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g., in the laminar flow hood.*
- *For further analysis or cell processing note that the volumes listed on Screen 7.11 are not the exact volumes but volumes within the safety requirements.*

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

To continue, press **ENT**.

The program automatically continues with the instructions to install the tubing set on the instrument.

▶ **Proceed to STEP 3.**

7.2.4 ENRICHMENT 3.2

IMPORTANT

- *The separation program ENRICHMENT 3.2 must only be used with the CliniMACS Tubing Set (REF 161-01).*
- *Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step. To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

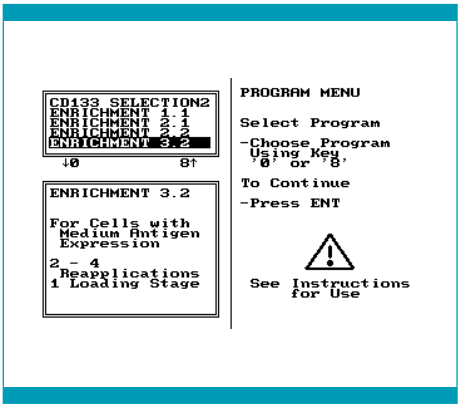
Choice of separation program ENRICHMENT 3.2

The window indicates Screen 7.12.

Choose ENRICHMENT 3.2.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys **0** and **8**.

To proceed with the highlighted program, press **ENT**.



Screen 7.12: Choice of separation program

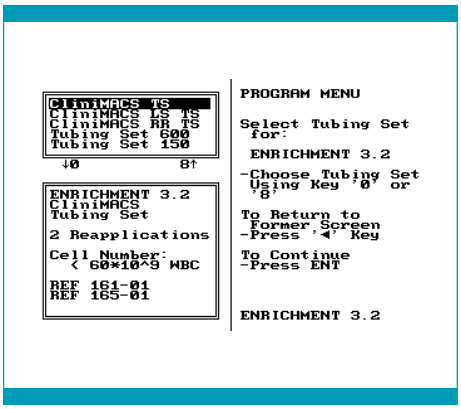
Selection of tubing set

The window indicates Screen 7.13.

Confirm the correct program has been chosen. If not, press ◄ to return to the previous step in order to amend the choice.

Select the tubing set by highlighting it in the box on the left-hand side with the black bar. Move the bar up and down using the keys 0 and 8.

The window below indicates the relevant data like capacity and catalogue number of the selected tubing set.



Screen 7.13: Selection of the tubing set

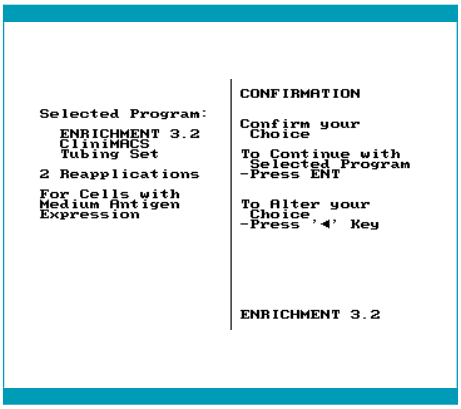
To continue with the selected combination of separation program and tubing set, press ENT.

Confirmation

The window indicates Screen 7.14.

To confirm the combination of separation program and tubing set and to proceed, press ENT.

If not, press ◄ to return to the previous step in order to amend the choice.



Screen 7.14: Confirmation

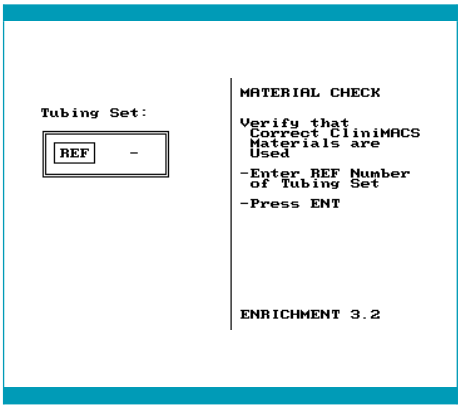
Material check

The window indicates Screen 7.15.

The query of the catalogue number (REF) of the tubing set serves as a security check to ensure that the selected tubing set can be used with the program selected.

Enter catalogue number of the selected tubing set and press ENT to proceed.

If a non-corresponding catalogue number has been entered, a message appears.



Screen 7.15: Material check

To confirm press ENT and enter the correct catalogue number. If the entered number is still wrong, the message appears a second time. After pressing ENT the program will return to the program menu (see Screen 7.12).

If the material check was successful, the program continues automatically with the instructions to install the tubing set.

▶ **Proceed to STEP 3.**

7.2.5 DEPLETION 2.1

IMPORTANT

- *The separation program DEPLETION 2.1 must only be used with the CliniMACS Tubing Set LS (REF 162-01).*
- *Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step. To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

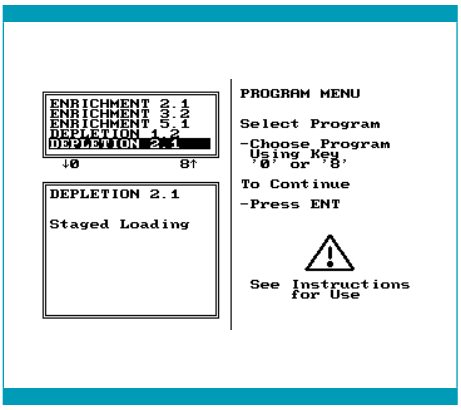
Choice of separation program DEPLETION 2.1

The window indicates Screen 7.16.

Choose DEPLETION 2.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys 0 and 8.

To proceed with the highlighted program, press ENT.



Screen 7.16: Choice of separation program

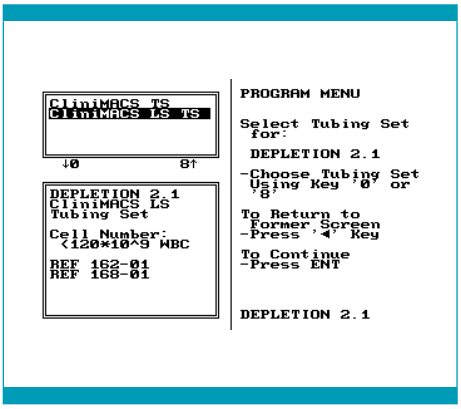
Selection of the tubing set

The window indicates Screen 7.17.

Confirm that the correct program has been chosen.

If not, press ◀ to return to the previous step in order to amend the choice.

Select the tubing set by highlighting it in the box at the top left-hand side with the black bar. Move the bar up and down by using the keys 0 and 8.



Screen 7.17: Selection of the tubing set

The window below indicates the relevant data like capacity and catalogue number of the selected tubing set.

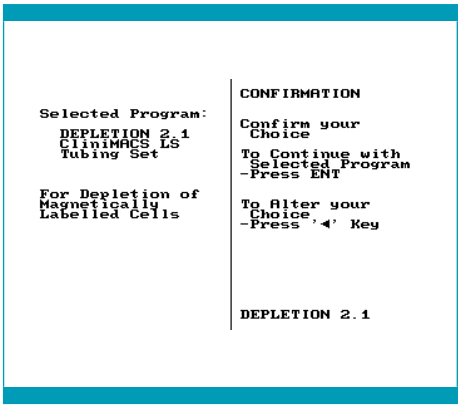
To continue with the selected combination of separation program and tubing set, press ENT.

Confirmation

The window indicates Screen 7.18.

To confirm the combination of separation program and tubing set and to proceed, press ENT.

If not, press ◄ to return to the previous step in order to amend the choice.



Screen 7.18: Confirmation

Material check

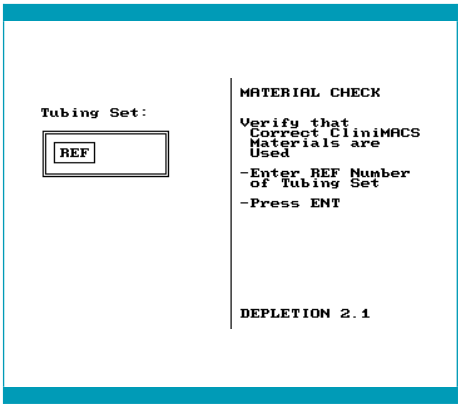
The window indicates Screen 7.19.

The query of the catalogue number (REF) of the tubing set serves as a security check to ensure that the tubing set the operator selected can be used.

Enter catalogue number of the selected tubing set and press ENT to proceed.

If a non-corresponding catalogue number has been entered, a message appears.

To confirm press ENT and enter the correct catalogue number. If the entered number is still wrong, the message appears a second time. After pressing ENT the program will return to the program menu (see Screen 7.16).

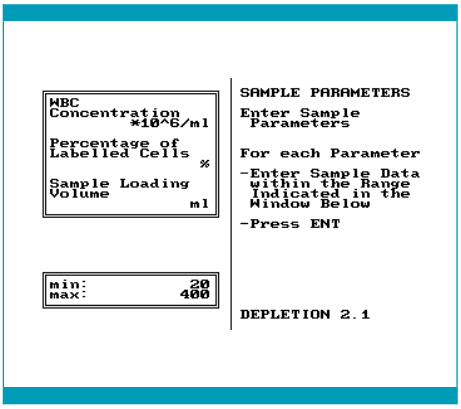


Screen 7.19: Material check

Sample parameter input

If the material check has been successful, the program continues automatically with the query for the sample parameters that are necessary to adjust the separation to each individual sample and to provide the operator with important information for required buffer and bag volumes.

The window indicates Screen 7.20.



Screen 7.20: Sample parameter input

1. Start with entering the WBC concentration of the sample. The allowed range is shown in the box on the bottom left-hand side. Press ◄ to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal.

   IMPORTANT

   All sample parameters required refer to the sample post cell labeling and washing procedure. The allowed range shown in the box on the bottom left-hand side always takes into account the previous input. After each input the software calculates and adjusts the accepted range of the next query.

   ⚠ CAUTION

   Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss. Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, this will result in increased processing time and enlarged volume of the target cell fraction. Always ensure to enter the correct parameters.

   To continue with the next input, press ENT.

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”).

   To continue with the next input, press ENT.

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”).

To continue, press ENT.

Calculation

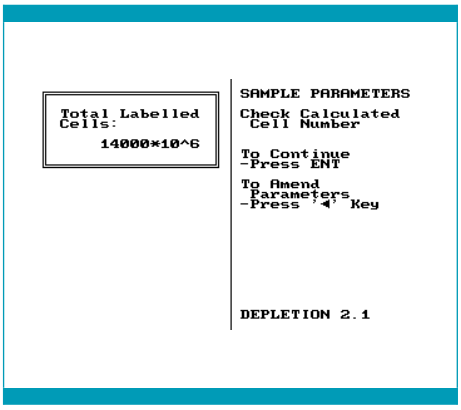
The CliniMACS Plus Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens:

- The result of the calculation is shown on a screen similar to Screen 7.21. Ensure the calculated number of labeled cells is correct.

  To continue, press **ENT**.

  A correction of the data is possible. Press ◄ to return to the previous screen for correction.

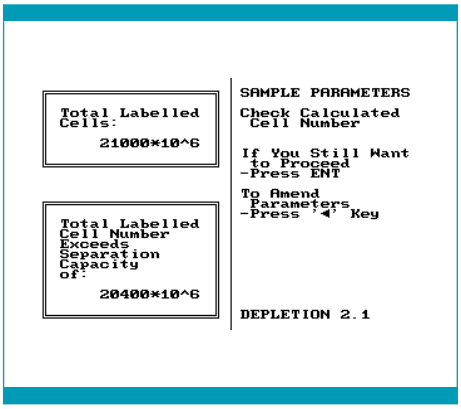


Screen 7.21: Calculation

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS Plus System a screen similar to Screen 7.22 is indicated. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press ◄ to return to the previous screen to correct the sample parameters.

  To avoid overloading the system, split the sample and determine the volume of each portion. Press ◄ to return to the previous Screen 7.20. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To continue, press **ENT**.



Screen 7.22: Calculation: Cell number exceeds separation capacity

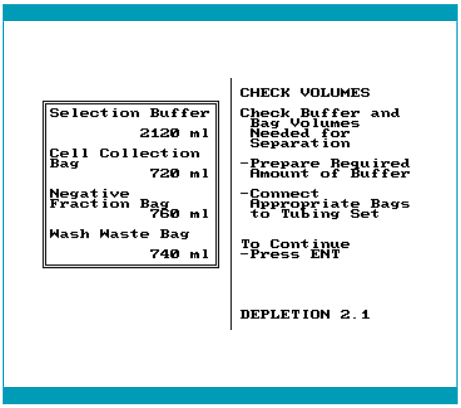
Volume information

From the total number of labeled cells (see section “Calculation”) the software calculates the number of separation stages, the amount of buffer needed for the entire separation and the liquid volumes that will be collected in Negative Fraction Bag, Buffer Waste Bag and Cell Collection Bag.

Standard values are the following:

- CliniMACS PBS/EDTA Buffer 1,000 mL
- Negative Fraction Bag 500 mL
- Buffer Waste Bag 500 mL
- Cell Collection Bag 150 mL

If one of the calculated values exceeds these standard values, Screen 7.23 appears to inform about the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set (capacity approximately 500 mL) by alternative bags of appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.



Screen 7.23: Check volumes

1. Confirm the requested amount of buffer is available. Do not attach more than three liters of buffer on the bag hanger.

   NOTICE

   Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 3 kg. Do not overload the bag hangers.

2. Open the tray of the tubing set under sterile conditions and replace the original bags with larger ones if necessary. Ensure unrestricted flow to these bags.

3. Attach a Cell Collection Bag of sufficient size to the free luer connector. If not specified on Screen 7.23, the maximum elution volume of the target cell fraction is 150 mL. Ensure that unrestricted flow to the Cell Collection Bag is possible.

   IMPORTANT

   - *Ensure the required amount of buffer is available and the volume of the bags mentioned on Screen 7.23 is sufficient. Otherwise the performance of the separation will be compromised.*
   - *Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g., in the laminar flow hood.*
   - *For further analysis or cell processing note that the volumes listed on Screen 7.23 are not the exact volumes but volumes within the safety requirements.*

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

To continue, press **ENT**.

The program automatically continues with the instructions to install the tubing set on the instrument.

▶ **Proceed to STEP 3.**

7.2.6 DEPLETION 3.1

IMPORTANT

- *The separation program DEPLETION 3.1 must only be used with the CliniMACS Depletion Tubing Set (REF 261-01).*
- *Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step. To correct a mistake during data input, press the "Undo" key (see section "Description" in the CliniMACS Plus Instrument User Manual).*

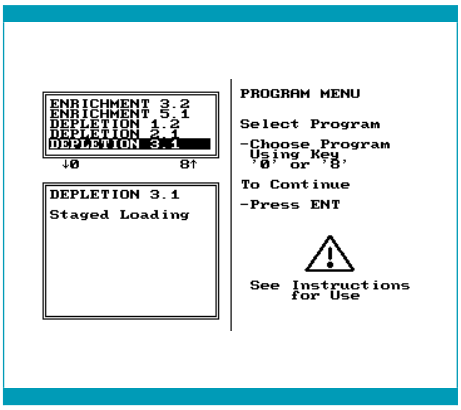
Choice of separation program DEPLETION 3.1

The window indicates Screen 7.24.

Choose DEPLETION 3.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys **0** and **8**.

To proceed with the highlighted program, press **ENT**.



Screen 7.24: Choice of separation program

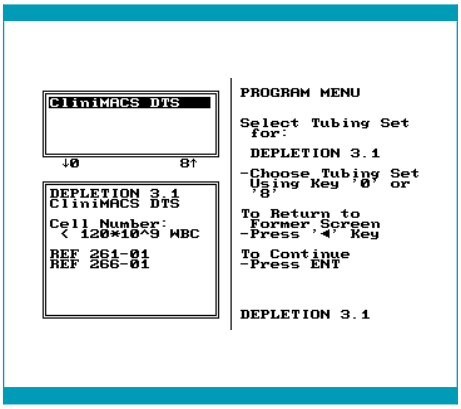
Selection of the tubing set

The window indicates Screen 7.25.

Confirm that the correct program has been chosen. If not, press ◄ to return to the previous step in order to amend the choice.

Select the tubing set by highlighting it in the box at the top left-hand side with the black bar. Move the bar up and down by using the keys **0** and **8**.

The window below indicates the relevant data like capacity and catalogue number of the selected tubing set.



Screen 7.25: Selection of the tubing set

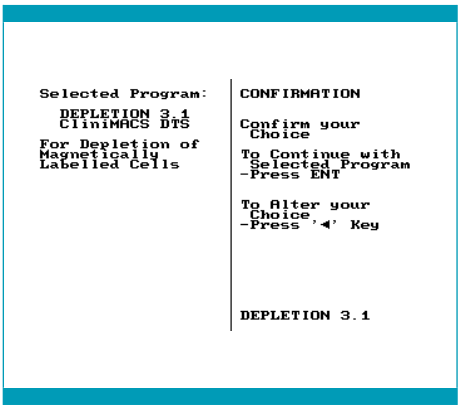
To continue with the selected combination of separation program and tubing set, press **ENT**.

Confirmation

The window indicates Screen 7.26.

To confirm the combination of separation program and tubing set and to proceed, press ENT.

If not, press ◄ to return to the previous step in order to amend the choice.



Screen 7.26: Confirmation

Material check

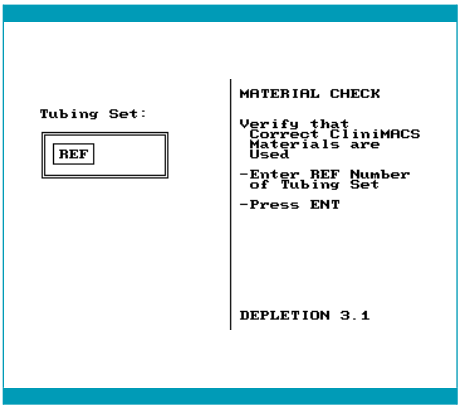
The window indicates Screen 7.27.

The query of the catalogue number (REF) of the tubing set serves as a security check to ensure that the tubing set the operator selected can be used.

Enter catalogue number of the selected tubing set and press ENT to proceed.

If a non-corresponding catalogue number has been entered, a message appears.

To confirm press ENT and enter the correct catalogue number. If the entered number is still wrong, the message appears a second time. After pressing ENT the program will return to the program menu (see Screen 7.25).

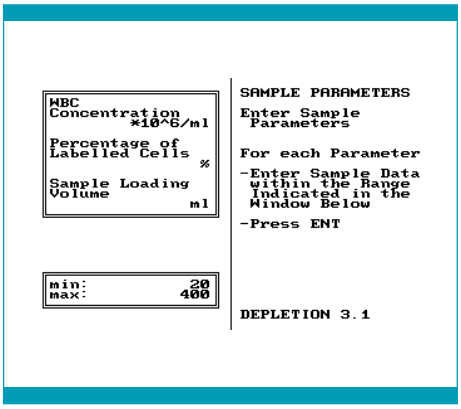


Screen 7.27: Material check

Sample parameter input

If the material check has been successful, the program continues automatically with the query for some sample parameters that are necessary to adjust the separation to each individual sample and to provide the operator with important information for required buffer and bag volumes.

The window indicates Screen 7.28.



Screen 7.28: Sample parameter input

1. Start with entering the WBC concentration of the sample. The allowed range is shown in the box on the bottom left-hand side. Press ◄ to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal.

   IMPORTANT

   All sample parameters required refer to the sample post cell labeling and washing procedure. The allowed range shown in the box on the bottom left-hand side always takes into account the previous input. After each input the software calculates and adjusts the accepted range of the next query.

   ⚠ CAUTION

   Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss. Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, this will result in increased processing time and enlarged volume of the target cell fraction. Always ensure to enter the correct parameters.

   To continue with the next input, press ENT.

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”).

   To continue with the next input, press ENT.

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see “Important”).

To continue, press ENT.

Calculation

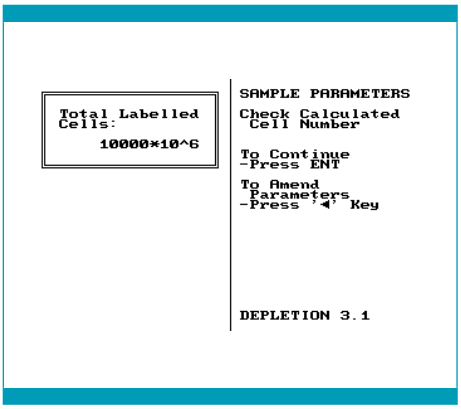
The CliniMACS Plus Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens:

- The result of the calculation is shown on a screen similar to Screen 7.29. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT.

  A correction of the data is possible. Press ◄ to return to the previous screen for correction.

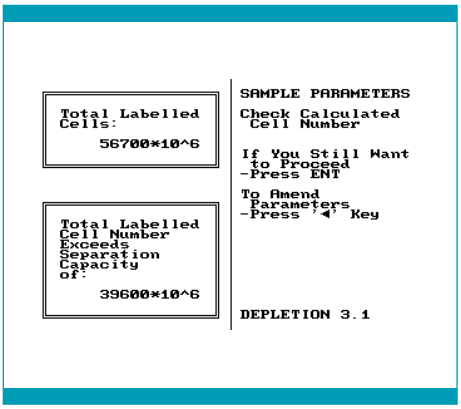


Screen 7.29: Selection of the tubing set

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS Plus System a screen similar to Screen 7.30 is indicated. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press ◄ to return to the previous screen to correct the sample parameters.

  To avoid overloading the system, split the sample and determine the volume of each portion. Press ◄ to return to the previous Screen 7.28. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To continue, press ENT.



Screen 7.30: Calculation: Cell number exceeds separation capacity

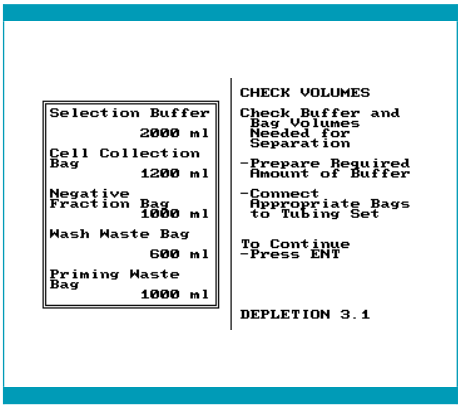
Volume information

From the total number of labeled cells (see section “Calculation”) the software calculates the number of separation stages, the amount of buffer needed for the entire depletion and the liquid volumes that will be collected in the Cell Collection Bag, Non-Target Cell Bag, and Reapplication Bag.

IMPORTANT

- *Negative Fraction Bag corresponds to Non-Target Cell Bag.*
- *Priming Waste Bag corresponds to Reapplication Bag.*

If one of the calculated values exceeds these standard values, a screen similar to Screen 7.31 appears to inform about the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set with alternative bags of appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.



Screen 7.31: Check volumes

1. Confirm the requested amount of buffer is available. Do not attach more than three liters of buffer on the bag hanger.

   NOTICE

   Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 3 kg. Do not overload the bag hangers.

2. Open the tray of the tubing set under sterile conditions and replace the original bags with larger ones if necessary. Ensure unrestricted flow to these bags.

3. If replacement is necessary, replace the Cell Collection Bag of the tubing set with a transfer bag of sufficient size. Determine the weight of the new, empty Cell Collection Bag and record it. Ensure that unrestricted flow to the Cell Collection Bag is possible. Alternatively, attach an additional 600 mL transfer bag to the first Cell Collection Bag, e.g., by using a plasma transfer set.

   IMPORTANT

   - *Ensure the required amount of buffer is available and the volume of the bags mentioned on Screen 7.31 is sufficient. Otherwise the performance of the separation will be compromised.*
   - *Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g., in the laminar flow hood.*
   - *For further analysis or cell processing note that the volumes listed on Screen 7.31 are not the exact volumes but volumes within the safety requirements.*

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

To continue, press **ENT**.

The program automatically continues with the instructions to install the tubing set on the instrument.

▶ **Proceed to STEP 3.**

7.3 STEP 3: Installation of CliniMACS Tubing Sets

7.3.1 CliniMACS Tubing Set and CliniMACS Tubing Set LS

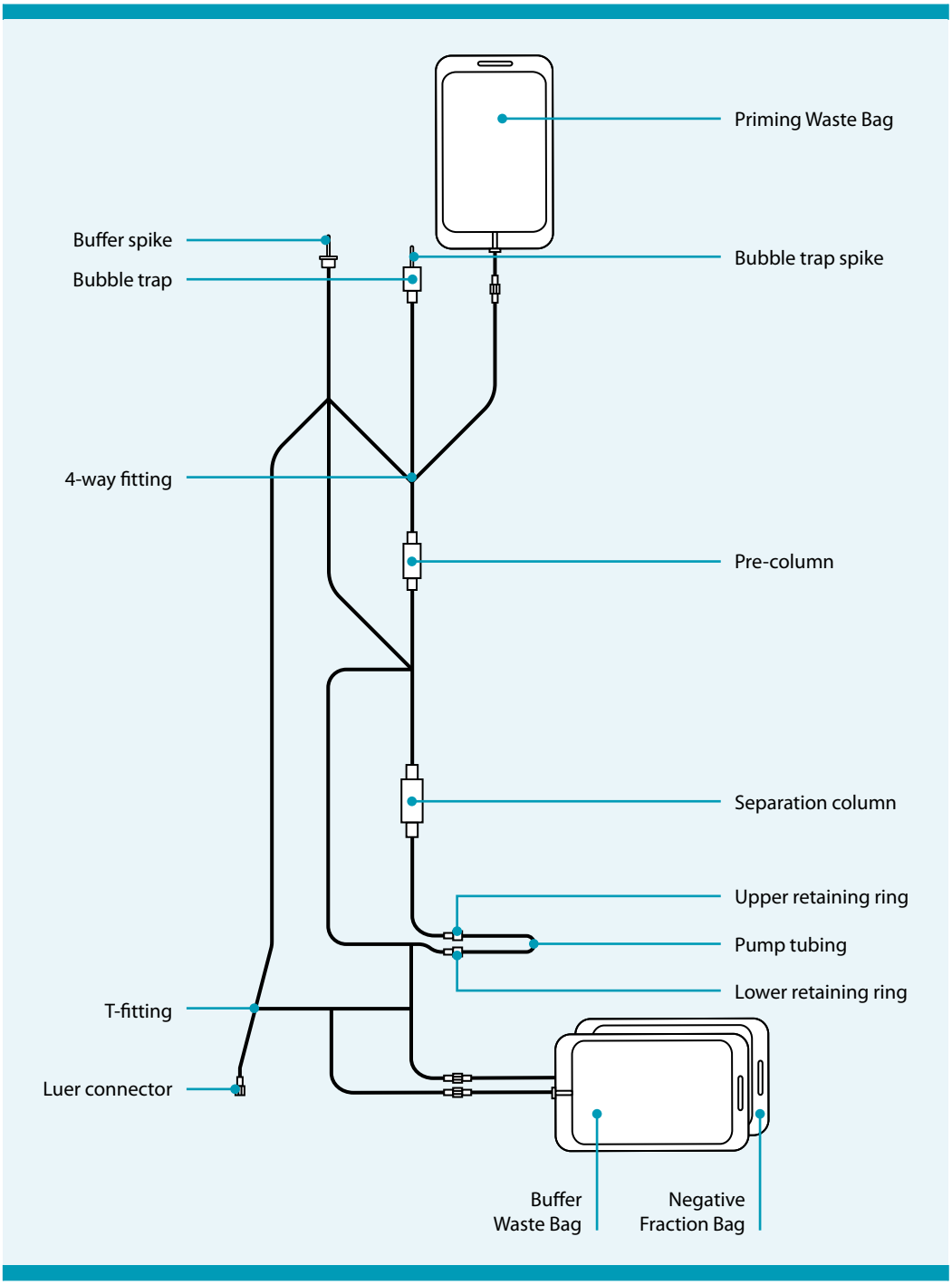
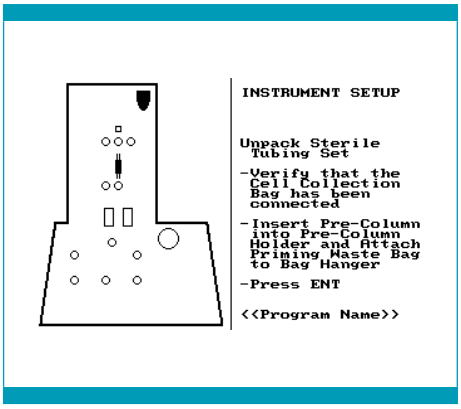


Figure 7.2: General construction of a CliniMACS Tubing Set (e.g., CliniMACS Tubing Set)

Preparation for tubing set installation

The window indicates Screen 7.32.

The instruction is shown on the right, and a diagram corresponding to the instruction on the left side of the screen. The blinking features on the screen indicate the areas of attention.



Screen 7.32: Unpack tubing set

The CliniMACS Tubing Set and the CliniMACS Tubing Set LS are each provided in sealed, sterilized packages. Each tubing set contains preassembled tubing and columns for one cell separation (see Figure 7.2). When the packaging is intact, a sterile fluid path is provided.

⚠ WARNING

Risk of contamination. Damaged packaging indicates that the tubing set may no longer be sterile and therefore must not be used. Carefully inspect the packaging for damage, puncture or tears. Use the tubing set only if package is undamaged and sealed.

Note: The CliniMACS Plus Instrument shows the chosen program name, e.g., CD34 SELECTION 1, in the bottom line of the instrument screen. At any step during the tubing set installation the “Undo” key (see section “Description” in the CliniMACS Plus Instrument User Manual) can be pushed to return to the previous step.

1. Record the lot number and use-by date of the tubing set.

   Unpack the sterile tubing set under sterile conditions (e.g., laminar flow hood). Remove the pinch clamp used for packaging of the CliniMACS Tubing Set LS before usage of the tubing set. The clamp is labeled with “Remove before assembly to CliniMACS Plus Instrument”.

2. Check luer lock connections to bags. Luer locks must be closed tightly.

Connect Cell Collection Bag

1. Note the weight of the empty Cell Collection Bag.
2. In an aseptic environment, remove caps and attach the sterile Cell Collection Bag equipped with a Luer/Spike Interconnector on the tubing set before loading the tubing set onto the CliniMACS Plus Instrument. Ensure that the original tubing of the Cell Collection Bag is either clamped or sealed off.

   If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Make sure that all connections are closed tightly.
3. Ensure that unrestricted flow to the Cell Collection Bag is possible.
4. Proceed with the installation of the tubing set using either the procedure described below or the "Alternative installation for the CliniMACS Tubing Sets".

Attach Priming Waste Bag and insert pre-column

The window indicates Screen 7.32.

1. Attach the Priming Waste Bag to the right-hand bag hanger on the instrument (see Figure 7.3).
2. Adjust the height of the bag hanger. Raise or lower the bag hanger to accommodate the height to the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched.
3. Place the pre-column into the holder (see Figure 7.3).

To proceed, press **ENT**.

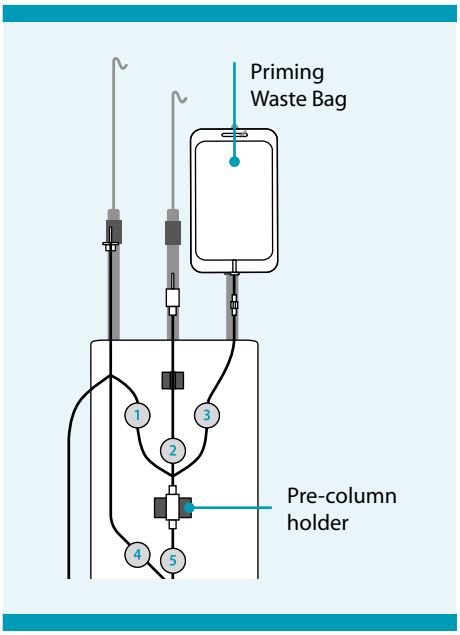


Figure 7.3: Attach Priming Waste Bag to bag hanger

Insert separation column and load valve no. 5

The window indicates Screen 7.33.

The valves shown in black on the screen will be opened automatically to facilitate tubing insertion.

1. Insert the separation column into the separation column holder (see Figure 7.4).

   ⚠ WARNING

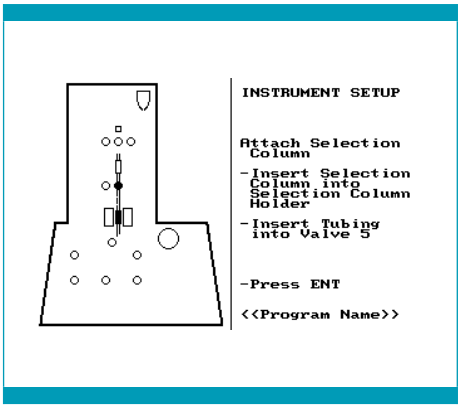
   Risk of injury. If the separation column is inserted or removed while the magnet unit is switched on, there is the risk of personal injury. Only insert or remove the separation column when the magnet unit is switched off.

2. Load the tubing into valve no. 5.

IMPORTANT

- *As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.*
- *Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.*
- *If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see Figure 7.5).*

To proceed, press **ENT**.



Screen 7.33: Insert separation column and load valve no. 5

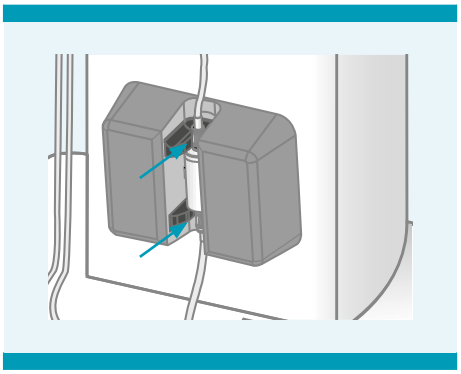


Figure 7.4: Separation column in separation column holder

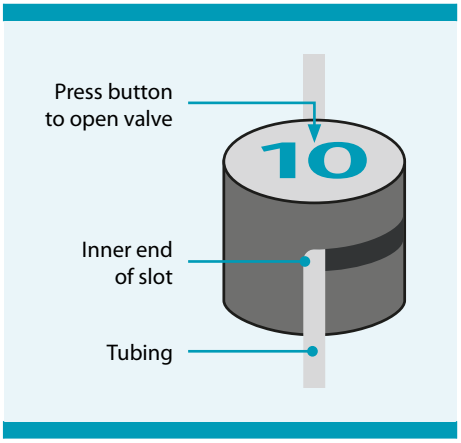


Figure 7.5: Correctly inserted tubing

Load valves nos. 1, 2, 3, and 4

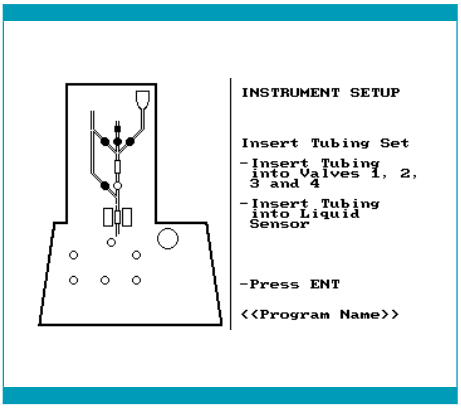
The window indicates Screen 7.34.

1. Load the tubing into valve no. 4. Confirm that the tubing is placed securely in the valve opening (see Figure 7.5). Pay particular attention to the area between valves nos. 4 and 5 (see Figure 7.6) and keep the four-way fitting upright.
2. Insert the tubing into valve no. 1.
3. Position the 4-way fitting just below valve no. 2. Pay particular attention to the area below valve no. 2.
4. Insert the tubing into valve nos. 2 and 3.
5. Mount the tubing between valve no. 2 and the bubble trap into the liquid sensor. Confirm that the tubing is placed correctly into the sensor fitting.

   IMPORTANT

   To ensure proper operation, both the liquid sensor and the tubing being inserted must be dry. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press **ENT**.



Screen 7.34: Load valves nos. 1, 2, 3, 4, and liquid sensor

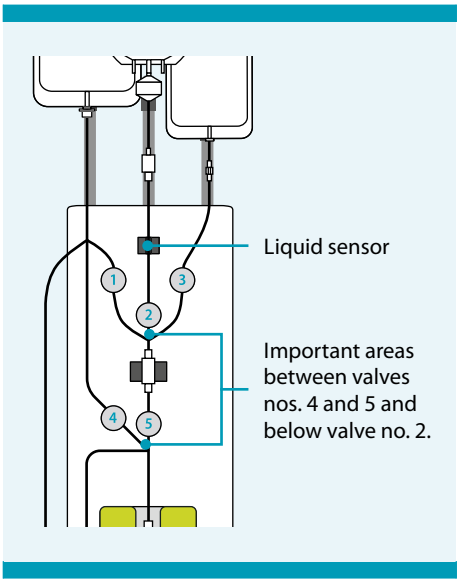
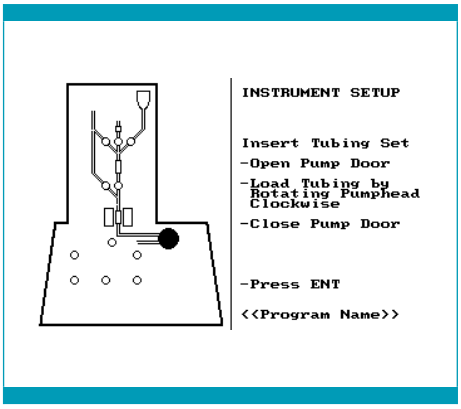


Figure 7.6: Tubing in valves

Load pump tubing

The window indicates Screen 7.35.



Screen 7.35: Load pump tubing

1. Open the pump door by lifting up at the left-hand edge.
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove on the pump housing (see Figure 7.7).
3. Rotate the pump roller clockwise until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise.
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing.
5. Rotate the pump roller clockwise and anticlockwise, to ensure that the pump roller moves freely.
6. Close the pump door.

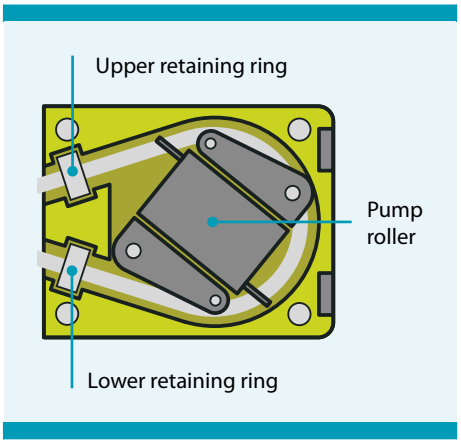


Figure 7.7: Load pump tubing

IMPORTANT

During the cell separation program, the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

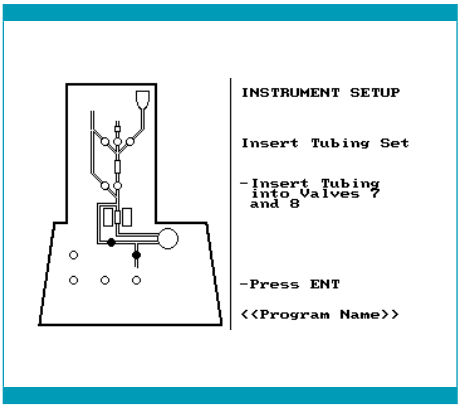
To proceed, press **ENT**.

Load valves nos. 7 and 8

The window indicates Screen 7.36.

1. Load the tubing into valve no. 7.
2. Load the tubing into valve no. 8.

To proceed, press **ENT**.



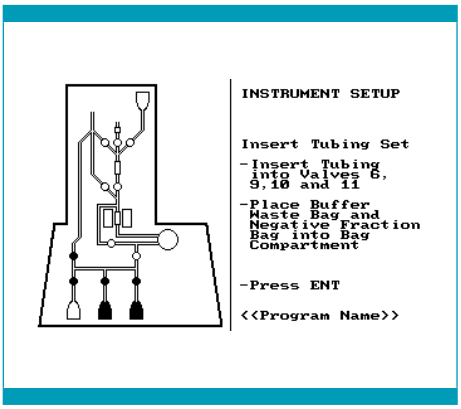
Screen 7.36: Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

The window indicates Screen 7.37.

1. Load the tubing into valves nos. 6, 9, 10, and 11.
2. Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid.

To proceed, press **ENT**.



Screen 7.37: Load valves nos. 6, 9, 10, and 11

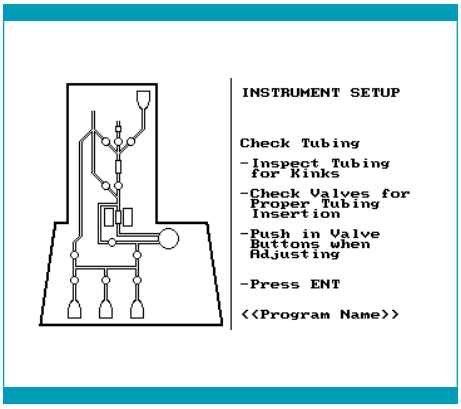
Recheck all tubing and attachments

The window indicates Screen 7.38.

Before beginning the cell separation, recheck all tubing and attachments.

Note: Check all valves for proper tubing insertion. Make sure that the tubing is spaced uniformly, and that there are no kinks or stretched areas in the tubing. Pay particular attention to the pre-column area, as well as the area between the pump and valves nos. 7 and 8 (see Figure 7.8) and between valves nos. 4 and 5 (see Figure 7.6). If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve. If a tubing has been adjusted, it is absolutely necessary to press the corresponding valves firmly two times.

To proceed, press ENT.



Screen 7.38: Recheck all tubing and attachments

Seating of valves

The window indicates Screen 7.39.

In order to ensure the proper fit of tubing in the valves, the instrument will operate all of the valves in sequence, twice. Watch and listen to make sure all valves are working properly. If any valve does not operate correctly, see chapter 8 “Troubleshooting” on page 205. This step can be repeated by pressing ◄ followed by ENT (see section “Description” in the CliniMACS Plus Instrument User Manual).

The magnet drive will also be tested during this check sequence.

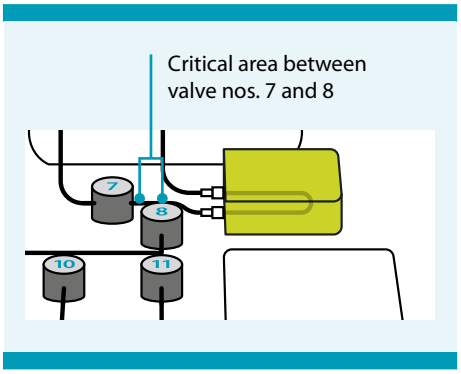
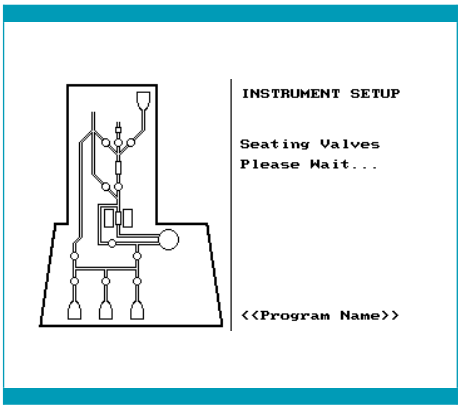


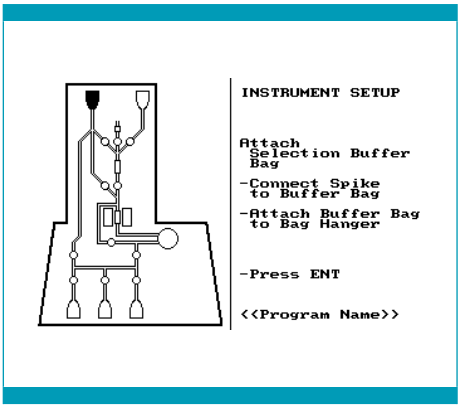
Figure 7.8: Tubing in valves



Screen 7.39: Seating of valves

Attach CliniMACS PBS/EDTA Buffer

The window indicates Screen 7.40.



Screen 7.40: Attach buffer bag

The prescribed buffer for CliniMACS Plus Separations is CliniMACS PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic techniques, remove the cap from the buffer spike on the tubing set (see Figure 7.2 on page 150) and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

   If more than one liter of buffer is necessary for a separation, connect two buffer bags using a plasma transfer set.

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see Figure 7.9).

3. Adjust the height of the buffer bag hanger. Raise or lower the bag hanger to accommodate the height to the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched.

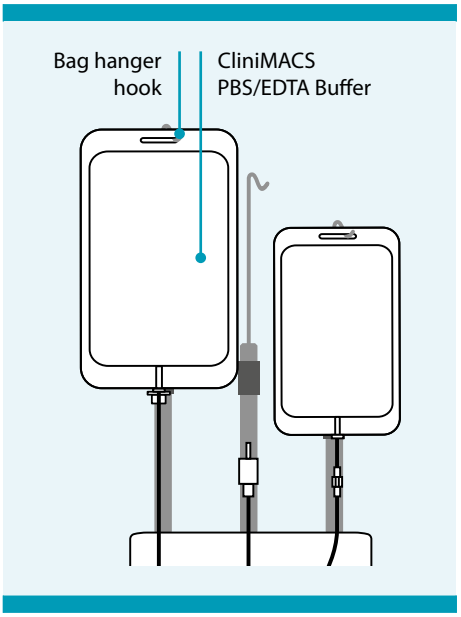


Figure 7.9: Attach buffer bag

NOTICE

Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 3 kg. Do not overload the bag hangers.

To proceed, press **ENT**.

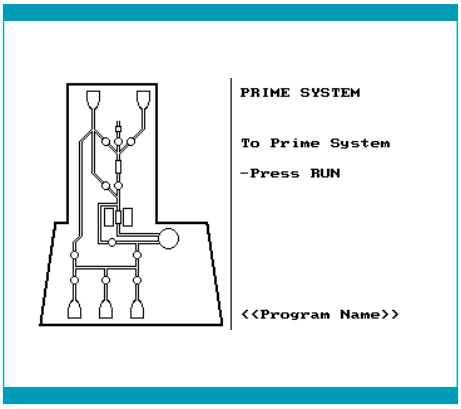
Start priming

The window indicates Screen 7.41.

To start priming, press **RUN**.

The window indicates Screen 7.42.

During the priming phase the tubing set is filled with buffer. The buffer will be circulated through the tubing set including both, the pre-column and the separation column. Priming waste is collected in the Priming Waste Bag and the Buffer Waste Bag (see Figure 7.2 on page 150). The priming cycles will continue, repeating a series of steps. The priming phase will take approximately 1 minute. Priming status will be updated on the display.



Screen 7.41: Start priming

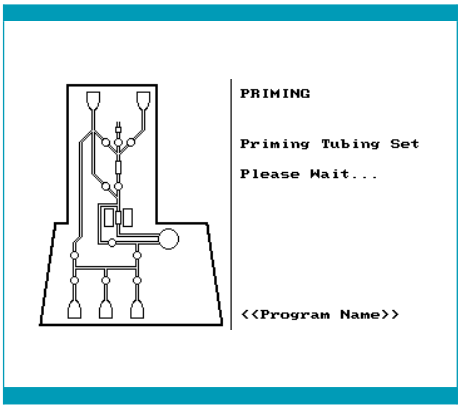
Check during priming

During the priming phase, check all tubing, fittings, valves, and columns for the appearance of any leaks or the presence of any folds that may block fluid flow.

If leaks or malfunctions are observed, press **STOP** to stop the run.

The operator will have 600 seconds to resolve the problem.

Resume the process by pressing **RUN**.



Screen 7.42: Priming in process

After 600 seconds, the separation will be aborted. If the operator cannot resolve the problem or if the tubing set is defective, remove the tubing set and replace it with a new one.

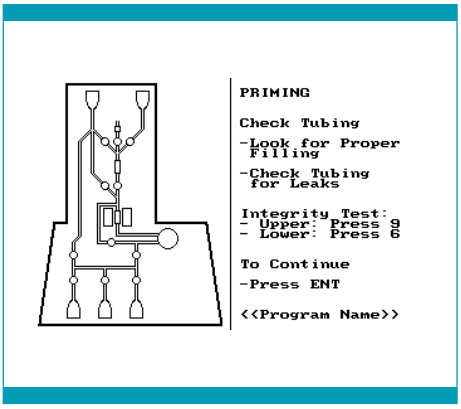
Note: Once priming has started, it is not possible to return to the instrument set-up procedure.

Final check of all tubing and attachments

The window indicates Screen 7.43.

Before continuing the program, check the following:

- fluid in all parts of tubing set (except the pre-system filter)
- no excess air in tubing set
- fluid in the Priming Waste Bag and the Buffer Waste Bag
- no fluid in the Negative Fraction Bag or in the Cell Collection Bag



Screen 7.43: Final check of tubing

Do not press ENT yet.

Integrity test

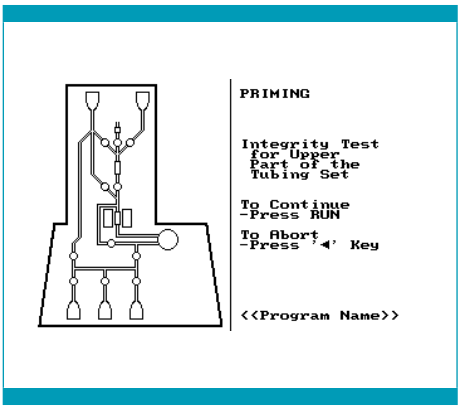
For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated subsequences, which allow both, the upper and the lower parts of the tubing set to be pressurized and observed separately.

Integrity test for the upper part of the tubing set

1. When the operator performs “Final check of all tubing and attachments” the window indicates Screen 7.43.
2. After performing the “Final check of all tubing and attachments”, continue with execution of the integrity test. **Do not** press ENT.
3. To execute the integrity test for the upper part, press 9.

   The window indicates Screen 7.44.
4. To start the test sequence, press RUN.

   To return to Screen 7.43, press ◄.



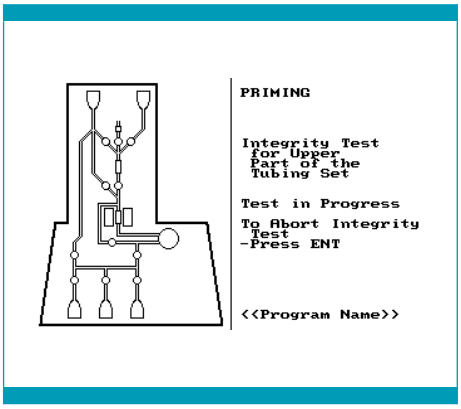
Screen 7.44: Start integrity test for upper part

5. Once RUN has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window indicates Screen 7.45.

   Overpressure will be created and held for two minutes. During this time the operator should watch all the connections above the upper pump tubing connection.

   At each point the test sequence can be finished by pressing ENT.



Screen 7.45: Integrity test for upper part in process

6. After two minutes the pressure is automatically released, and the window indicates Screen 7.43.

   Check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

7. If no leaks are observed, continue with the integrity test of the lower part of the tubing set.

Integrity test for the lower part of the tubing set

The window indicates Screen 7.46.

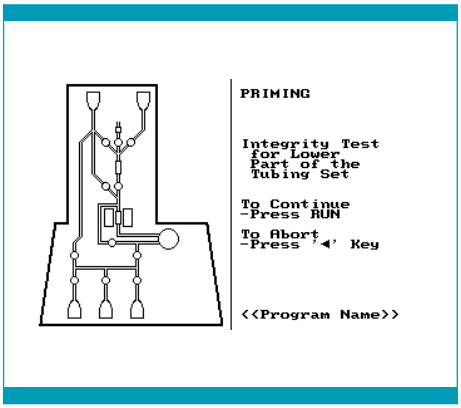
1. To enter the integrity test for the lower part, press 6.
   Do not press ENT.

   The window indicates Screen 7.46.

2. To start the test sequence, To start the test sequence, press RUN.

   To return to Screen 7.43, press ◄.

3. Once RUN has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

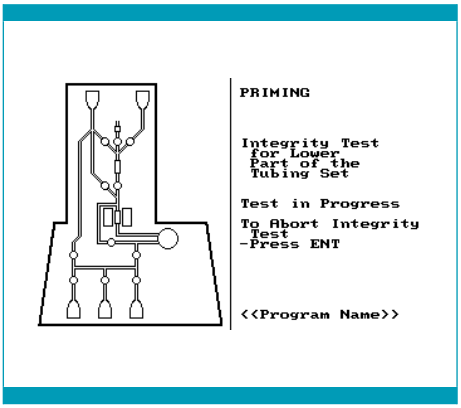


Screen 7.46: Start integrity test for lower part

The window indicates Screen 7.47.

Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tubing connection and the T-fittings between valves nos. 6, 8, 9, 10, and 11.

At each point the test sequence can be finished by pressing **ENT**.



Screen 7.47: Integrity test for lower part in process

4. After 30 seconds the pressure is automatically released, and the window indicates Screen 7.43.

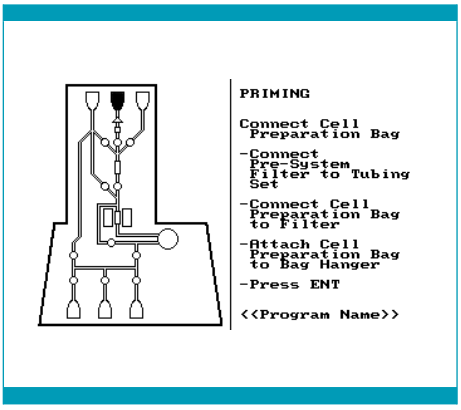
   Check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

5. If no leaks are observed the operator can now continue with the next step by pressing **ENT**.

Connect Cell Preparation Bag

The window indicates Screen 7.48.

Connect the Cell Preparation Bag containing the readily labeled cell product:



Screen 7.48: Connect Cell Preparation Bag

1. Remove the cap from the bubble trap spike of the tubing set (see Figure 7.2).
2. Remove the cap from the blunt end of the pre-system filter (see Figure 7.10). Firmly insert the spike into the pre-system filter.
3. Remove the cap from the pre-system filter spike.
4. Spike the pre-system filter into an unused port of the Cell Preparation Bag ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.
5. Carefully check the connection between the pre-system filter and the tubing set to confirm that the connection is secure.
6. Hang the Cell Preparation Bag on the center bag hanger.
7. Adjust the bag hanger for the Cell Preparation Bag to hold the Cell Preparation Bag in an upright position.

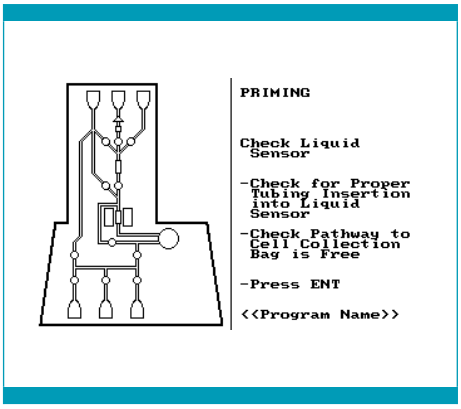
To proceed, press **ENT**.

Final check of the liquid sensor

The window indicates Screen 7.49.

1. Check the liquid sensor tubing. Ensure the tubing is properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.
2. Confirm that the unrestricted flow to the Cell Collection Bag is possible (e.g., open clamp below valve 9).

To proceed, press **ENT**.



Screen 7.49: Final check of the liquid sensor

▶ **Proceed to STEP 4.**

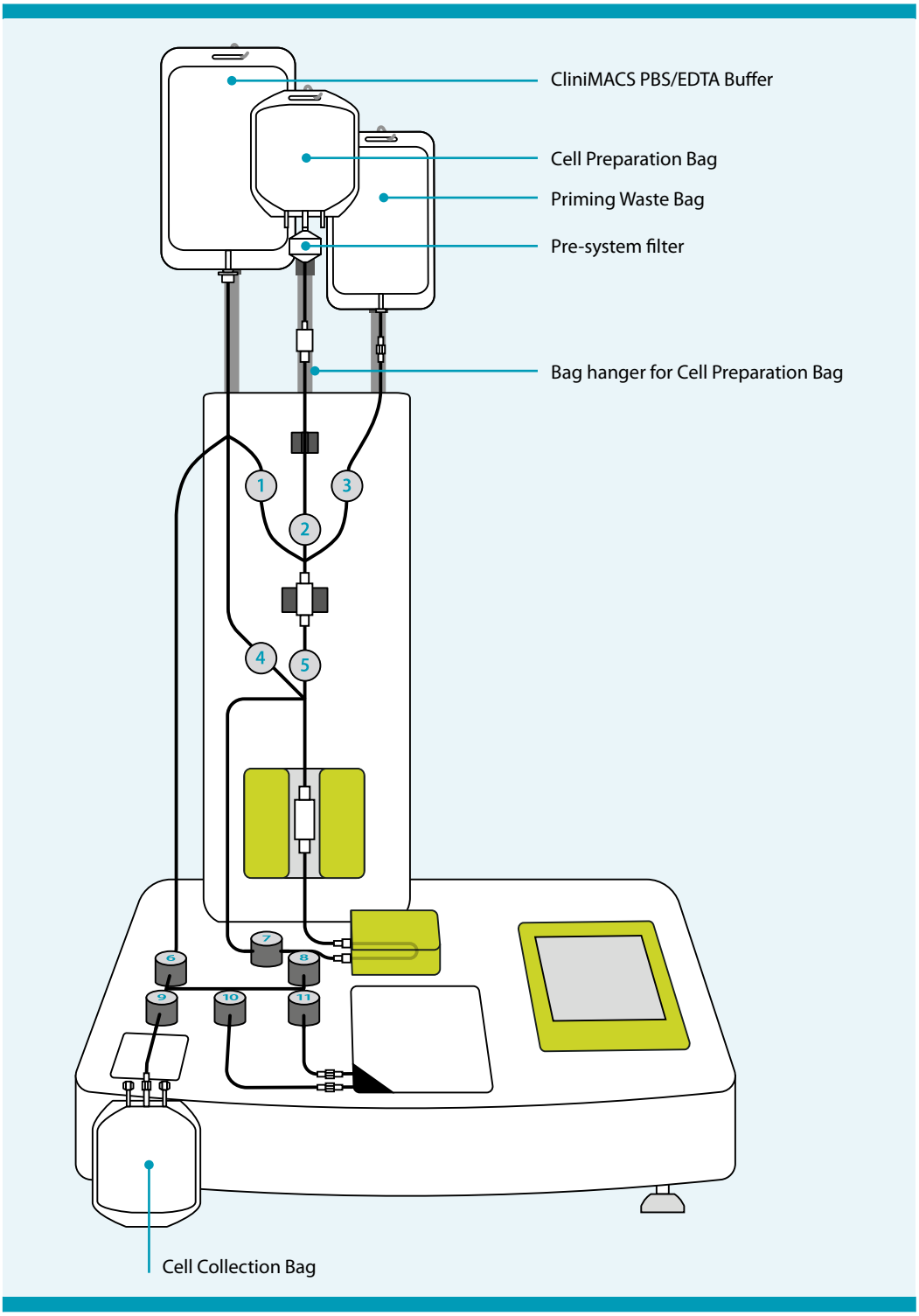


Figure 7.10: CliniMACS Plus Instrument with CliniMACS Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag

7.3.2 Alternative installation of CliniMACS Tubing Sets

The instructions in STEP 3 and the screens indicated by the CliniMACS Plus Instrument describe the installation of the CliniMACS Tubing Sets under sterile conditions (clean room).

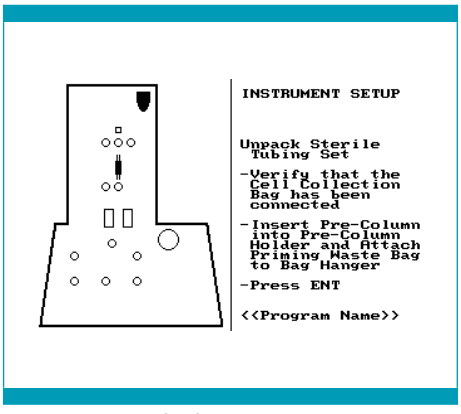
The CliniMACS Plus System itself is a closed system which does not necessarily need to be operated in a clean room. However, if operated outside a clean room, the installation procedure of the tubing set needs to be adapted in order to ensure that the sterility of the cell separation process is guaranteed.

The sterility of the cell separation process may be compromised during the attachment of the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag. To ensure that the system remains sterile, these components must be attached to the tubing set under sterile conditions (e.g., laminar flow hood). When the components are attached to the tubing set before installation onto the CliniMACS Plus Instrument, the order of the instructions provided by the instrument and the instructions in STEP 3 must be changed and further actions taken.

When operating the CliniMACS Plus Instrument outside a clean room, follow the following additional instructions, altering the instructions in STEP 3.

Preparation for tubing set installation

The window indicates Screen 7.50.



Screen 7.50: Unpack tubing set

As described, the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag must be attached to the tubing set before installing the tubing set onto the instrument under sterile conditions.

Unpack the tubing set under sterile conditions and attach the following components under sterile conditions:

- **Connect Cell Collection Bag**

  See page 152.

Attachment of CliniMACS PBS/EDTA Buffer

IMPORTANT

Before connecting the CliniMACS PBS/EDTA Buffer to the tubing set, clamp the tubing below the buffer spike with a locking forceps in order to prevent the buffer from flowing into the tubing set prior to installation (see clamp 1, Figure 7.11 on page 169).

Using aseptic techniques remove the cap from the buffer spike on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ensure that the spike has penetrated the bag.

Attachment of pre-system filter

Remove the cap from the spike of the bubble trap. Remove the cap from the lower opening of the pre-system filter.

Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter. Close the tubing just below the bubble trap using a locking forceps (see clamp 2, Figure 7.11 on page 169). This prevents the prepared cell suspension in the Cell Preparation Bag from entering pre-system filter and tubing set.

Attachment of Cell Preparation Bag

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells to the tubing set. Spike the Cell Preparation Bag with the pre-system filter ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ensure that the spike has penetrated the bag.

Installation on the CliniMACS Plus Instrument

Hang the buffer bag on the left bag hanger, the Cell Preparation Bag on the middle bag hanger and the Priming Waste Bag on the right bag hanger on the instrument.

To proceed, press **ENT**.

▶ **Attach Priming Waste Bag and insert pre-column**

See page 152.

▶ **Insert separation column and load valve no. 5**

See page 153.

▶ **Load valves nos. 1, 2, 3, and 4**

See page 154.

▶ **Load pump tubing**

See page 155.

▶ Load valves nos. 7 and 8

See page 156.

▶ Load valves nos. 6, 9, 10, and 11

See page 156.

▶ Recheck all tubing and attachments

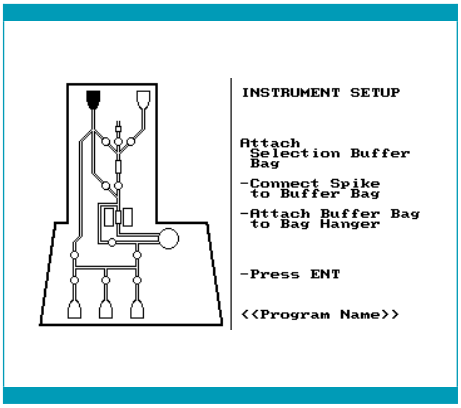
See page 157.

▶ Seating of valves

See page 157.

Attach CliniMACS PBS/EDTA Buffer

The window indicates Screen 7.51.



Screen 7.51: Attach buffer bag

1. The buffer bag was attached during “Preparation for tubing set installation”. Therefore only the height of the buffer bag hanger may need to be adjusted. Raise or lower the hanger to accommodate the size of the buffer bag, ensuring that the height allotted is high enough to prevent the tubing from severe bending that could restrict the liquid flow, and low enough to avoid stretching the tubing or connections.
2. Remove the locking forceps from the tubing just below the buffer spike.

To proceed, press **ENT**.

▶ Start priming

See page 159.

▶ Check during priming

See page 159.

▶ Final check of all tubing and attachments

See page 160.

▶ Integrity test

See page 160.

Connect Cell Preparation Bag (& pre-system filter)

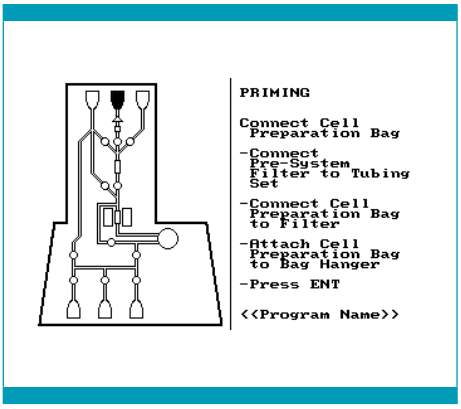
The window indicates Screen 7.52.

1. The Cell Preparation Bag and the pre-system filter were attached during "Preparation for tubing set installation".
2. Remove the locking forceps below the bubble trap.

To proceed, press **ENT**.

▶ Final check of the liquid sensor

See page 163.



Screen 7.52: Connect Cell Preparation Bag

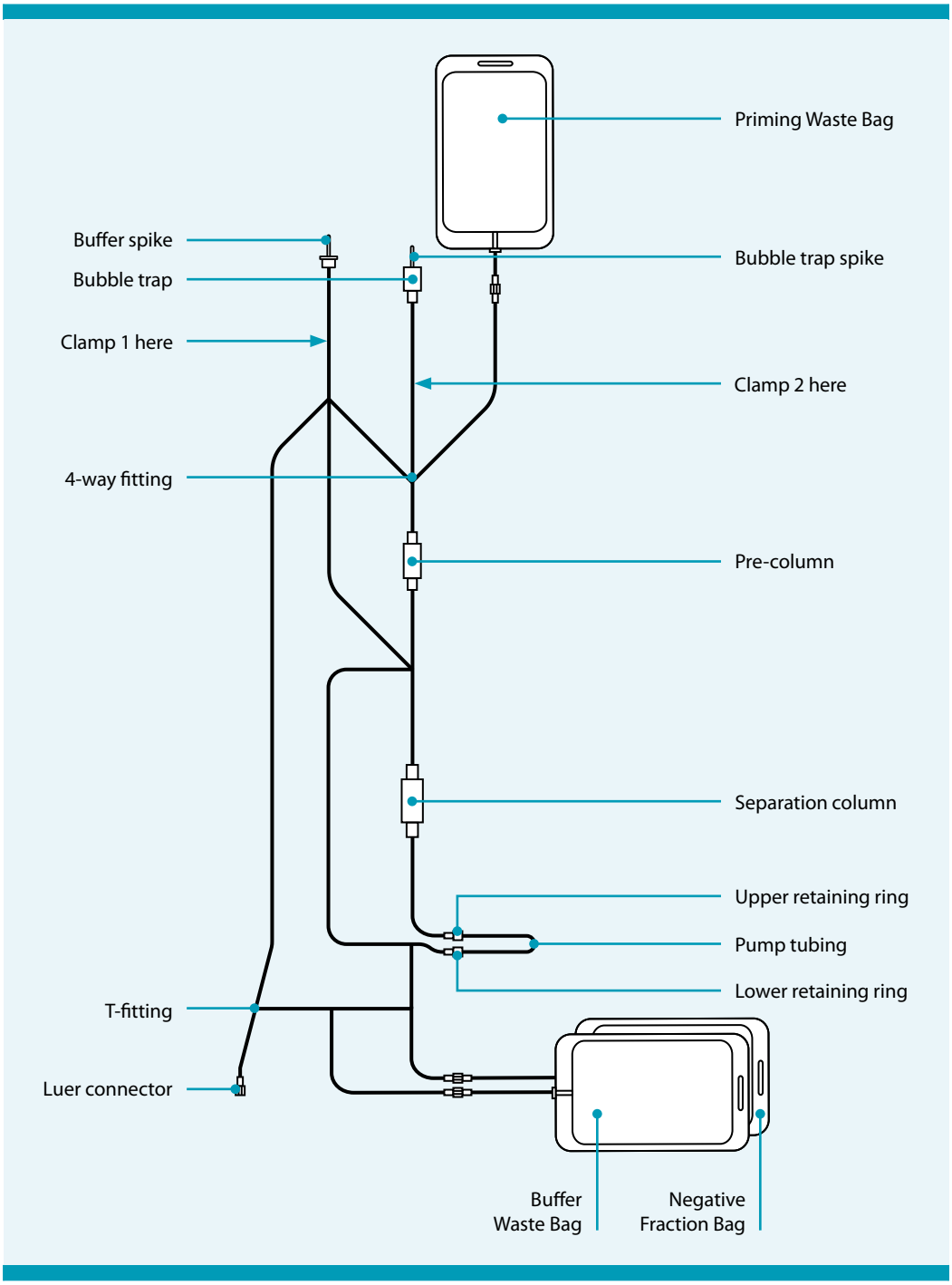


Figure 7.11: General construction of a CliniMACS Tubing Set (e.g., CliniMACS Tubing Set) with clamping positions 1 and 2

7.3.3 CliniMACS Depletion Tubing Set

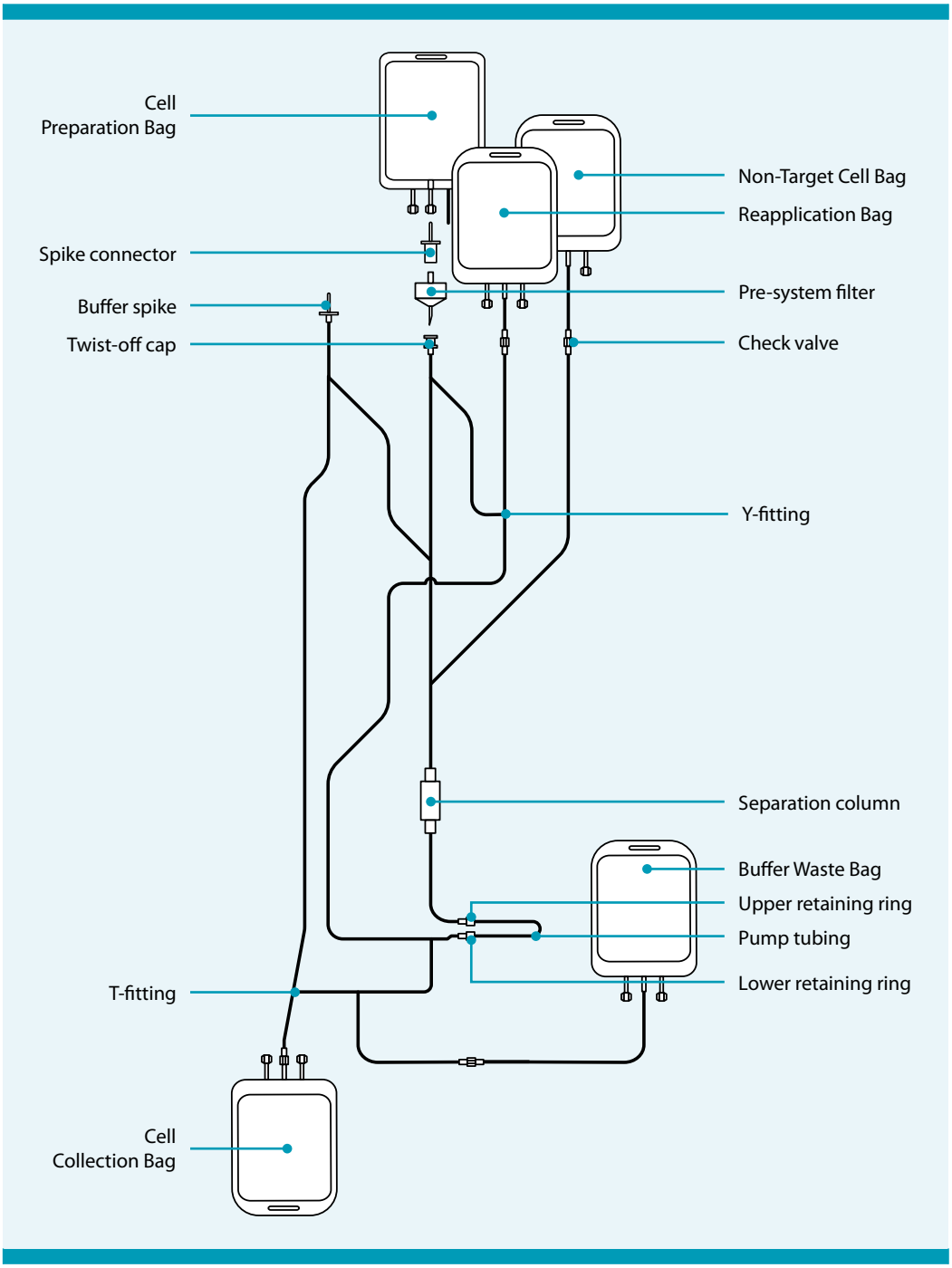
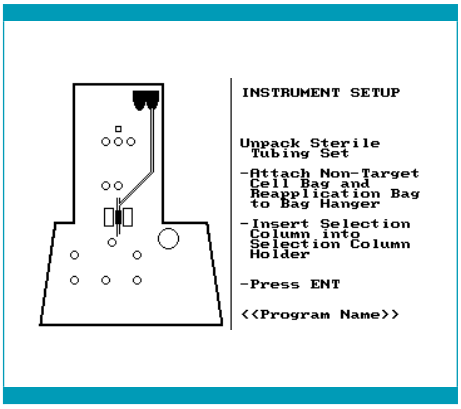


Figure 7.12: General construction of a CliniMACS Depletion Tubing Set

Preparation for tubing set installation

The window indicates Screen 7.53.



Screen 7.53: Unpack tubing set

The instruction is on the right and a diagram corresponding to the instruction is indicated on the left. The blinking features on the screen indicate the areas of attention.

The CliniMACS Depletion Tubing Set is provided in a sealed, sterilized package. Each tubing set contains preassembled tubings, column and bags for one cell separation (see Figure 7.12). When the packaging is intact, a sterile fluid path is provided.

Note: The CliniMACS Plus Instrument shows the chosen program name, e.g., DEPLETION 3.1, in the bottom line. At any step during the tubing set installation the “Undo” key (see section “Description” in the CliniMACS Plus Instrument User Manual) can be pushed to return to the previous step.

1. Record the lot number and use-by date of the tubing set.

   Unpack the sterile tubing set under sterile conditions (e.g., laminar flow hood). Remove the pinch clamp used for packaging before usage of the tubing set. The clamp is labeled with “Remove before assembly to CliniMACS Plus Instrument”.

2. Check luer lock connections to bags. Luer locks must be closed tightly.

Cell Collection Bag

The CliniMACS Depletion Tubing Set is provided with an attached Cell Collection Bag. Note the weight of the empty Cell Collection Bag. If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Make sure that all connections are closed tightly. Note the weight of the empty Cell Collection Bag(s).

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

1. Attach the Non-Target Cell Bag and the Reapplication Bag to the right hand bag hanger on the instrument (see Figure 7.13).
2. Adjust the height of the bag hangers. Raise or lower the bag hangers to accommodate the height to the size of the Non-Target Cell Bag and Reapplication Bag. Ensure that they are positioned high enough to prevent severe bending of the tubing that could restrict the flow and that it is low enough to avoid the tubing or connections being stretched.

   NOTICE

   Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 3 kg. Do not overload the bag hangers.
3. Insert the separation column into the separation column holder (see Figure 7.14).

   ⚠ WARNING

   Risk of injury. If the separation column is inserted or removed while the magnet unit is switched on, there is the risk of personal injury. Only insert or remove the separation column when the magnet unit is switched off.

To proceed, press ENT.

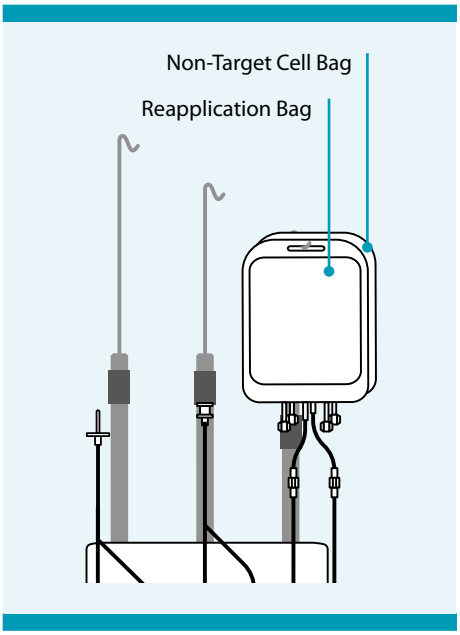


Figure 7.13: Attach Non-Target Cell Bag and Reapplication Bag

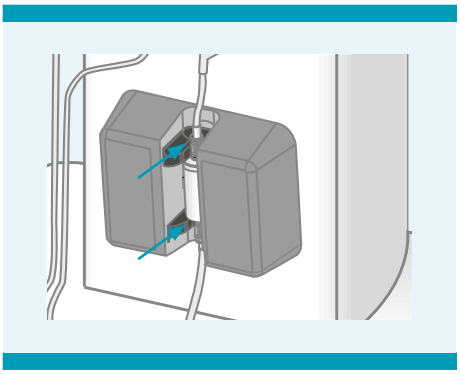


Figure 7.14: Separation column in separation column holder

Load valves nos. 1, 2, 3, 4, and 5

The window indicates Screen 7.54.

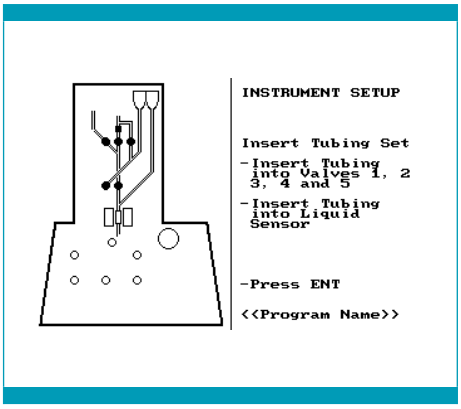
The valves shown on the screen will be opened automatically.

1. Load the tubing into valves nos. 1, 2, 3, 4, and 5.
2. Insert the tubing above valve no. 2 into the liquid sensor (see Figure 7.15).

IMPORTANT

- *To ensure proper operation, both the liquid sensor and the tubing being inserted must be dry. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.*
- *As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.*
- *Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.*
- *If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see Figure 7.16)*

To proceed, press **ENT**.



Screen 7.54: Load valves nos. 1, 2, 3, 4, and 5

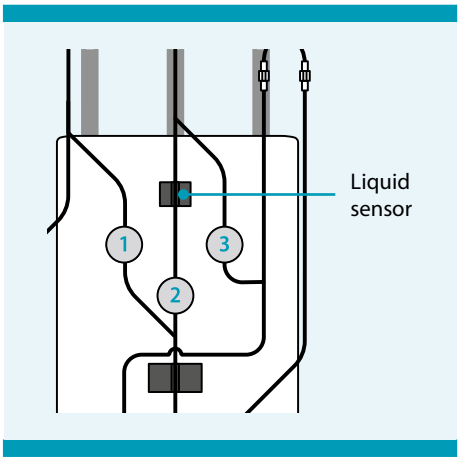


Figure 7.15: Tubing in valves

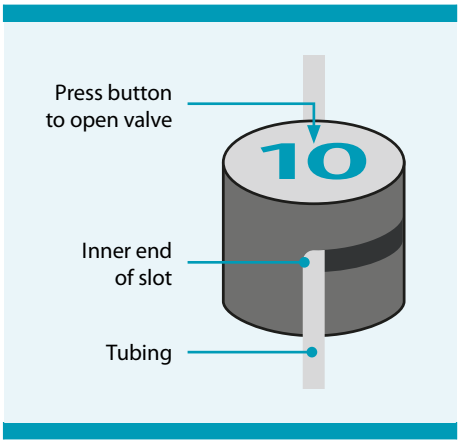
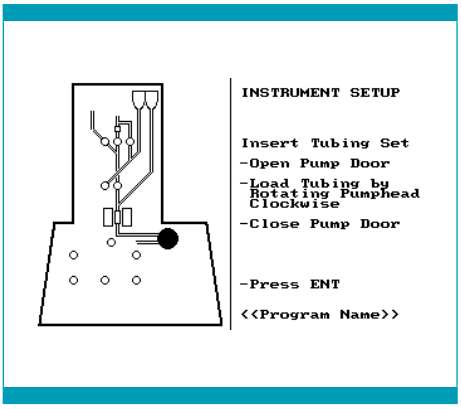


Figure 7.16: Correctly inserted tubing

Load pump tubing

The window indicates Screen 7.55.

1. Open the pump door by lifting up at the left-hand edge.
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove on the pump housing (see Figure 7.17).
3. Rotate the pump roller clockwise until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. (If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower ending and turning the pump roller anti-clockwise.)
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing.
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.



Screen 7.55: Load pump tubing

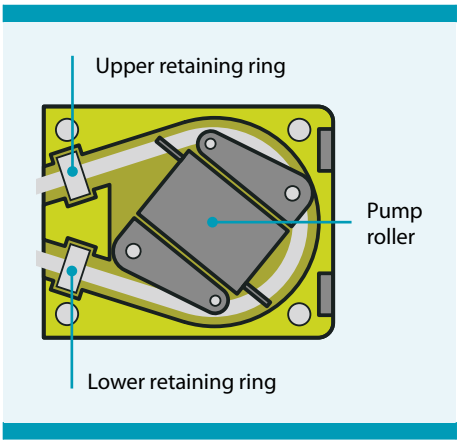


Figure 7.17: Load pump tubing

IMPORTANT

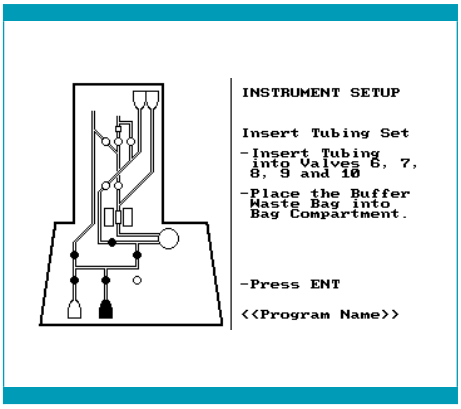
During the cell separation the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press **ENT**.

Load valves nos. 6, 7, 8, 9, and 10

The window indicates Screen 7.56.

1. Load the tubing into valve nos. 6, 7, 8, 9, and 10. Ensure that the tubing is placed securely in the valve opening.
2. Place the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid.
3. Ensure that unrestricted flow to the Cell Collection Bag is possible.



Screen 7.56: Load valves nos. 6, 7, 8, 9, and 10

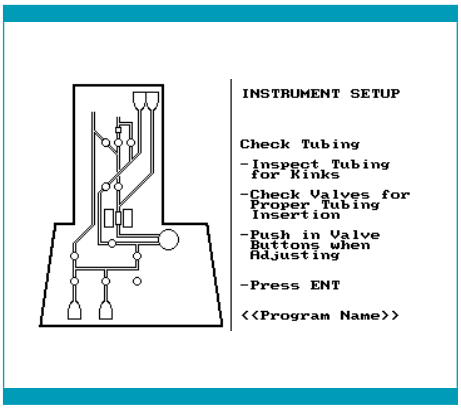
To proceed, press **ENT**.

Recheck all tubing and attachments

The window indicates Screen 7.57.

1. Beginning with valve no. 1, verify that the tubing fits properly and is positioned in each valve correctly.

   Reinspect the tubing in each valve. Ensure that the tubing enters and leaves each valve through the enlargement at the inner end of the slot (see Figure 7.16).
2. Check that the tubing is not kinked or twisted.



Screen 7.57: Recheck all tubing and attachments

Note: If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve.

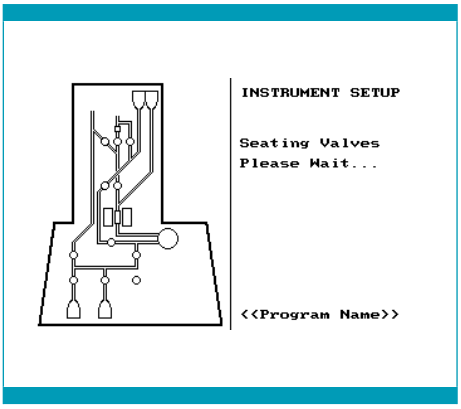
To proceed, press **ENT**.

Seating of valves

The window indicates Screen 7.58.

In order to ensure the proper fitting of tubing in the valves, the instrument will operate all of the valves in sequence twice. Watch and listen to make sure all valves are working properly. If any valve does not operate correctly, see chapter 8 “Troubleshooting” on page 205. This step can be repeated by pressing the ◄ followed by **ENT** (see section “Description” in the CliniMACS Plus Instrument User Manual).

The magnet drive will also be tested during this check sequence.



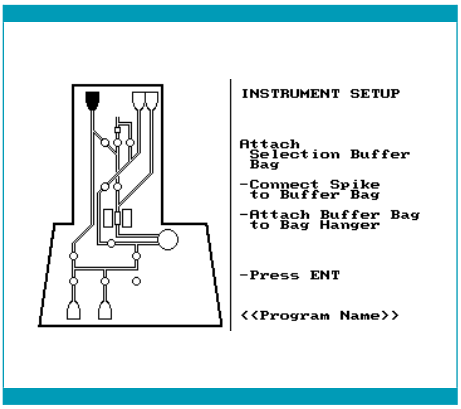
Screen 7.58: Seating of valves

Attach CliniMACS PBS/EDTA Buffer

The window indicates Screen 7.59.

The prescribed buffer for CliniMACS Plus Separations is CliniMACS PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic techniques, remove the cap from the buffer spike (see Figure 7.12 on page 170) on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ensure that the spike has penetrated the bag without external leakage.
2. Attach the buffer bag to the buffer bag hook on the bag hanger (see Figure 7.18).
3. Adjust the height of the buffer bag hanger. Raise or lower the bag hanger to accommodate the height to the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow and that it is low enough to avoid the tubing or connections being stretched.



Screen 7.59: Attach buffer bag

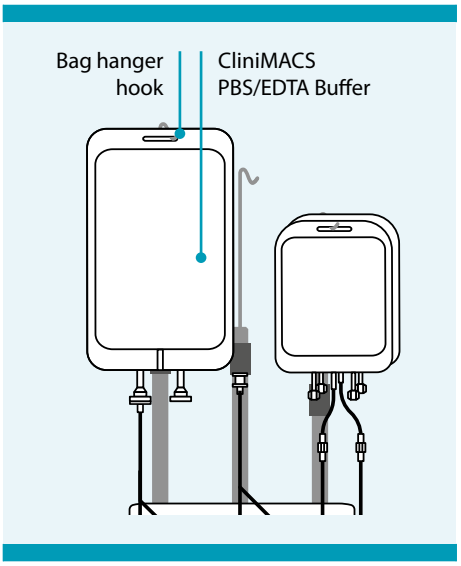


Figure 7.18: Attach buffer bag

IMPORTANT

Due to several gravimetric priming and rinsing steps, it is essential that the buffer bag is positioned as high as possible, ensuring that it is higher than the Reapplication Bag and the Non-Target Cell Bag (see Figure 7.20 on page 181).

To proceed, press **ENT**.

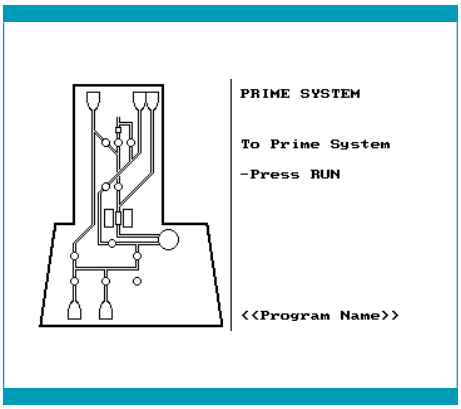
Start priming

The window indicates Screen 7.60.

To start priming, press **RUN**.

The window indicates Screen 7.61.

During the priming phase the tubing set is filled with CliniMACS PBS/EDTA Buffer. The buffer will be circulated through the tubing set including the separation column. Priming fluid is collected in the Buffer Waste Bag, Reapplication Bag and the Non-Target Cell Bag whereas the Cell Collection Bag remains empty (see Figure 7.12 on page 170). The priming cycles will continue, repeating a series of steps.



Screen 7.60: Start priming

The priming phase will take approximately 2.5 minutes. Priming status will be updated on the display.

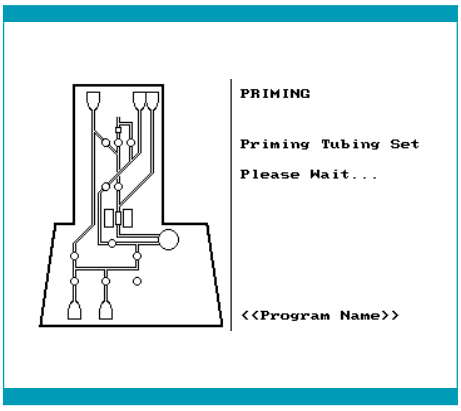
Check during priming

During the priming phase, check all tubing, fittings, valves and the separation column for the appearance of any leaks or the presence of any folds that may block fluid flow.

If leaks or malfunctions are observed, press **STOP** to stop the run.

The operator will have 600 seconds to resolve the problem.

Resume the process by pressing **RUN**.



Screen 7.61: Priming in process

After 600 seconds, the separation will be aborted. If the operator cannot resolve the problem or if the tubing set is defective, remove the tubing set and replace it with a new one.

Note: Once priming has started, it is not possible to return to the instrument set-up procedure.

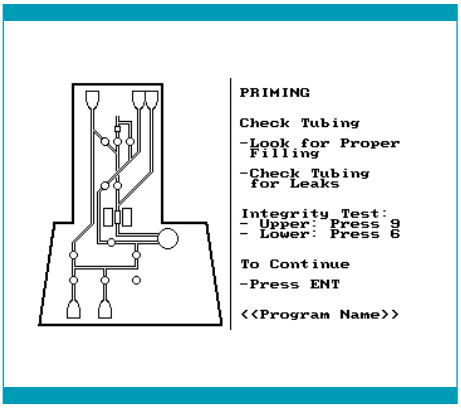
Final check of all tubing and attachments

The window indicates Screen 7.62.

Before beginning the run, check the following:

- fluid in all parts of tubing set except for tubing above valves nos. 2 and 3
- no excess air in tubing set
- fluid in Reapplication Bag, Buffer Waste Bag, and Non-Target Cell Bag
- no fluid in the Cell Collection Bag

Do not press ENT yet.



Screen 7.62: Final check of tubing and attachments

Integrity test

For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated sequences, which allow both the upper and the lower parts of the tubing set to be overpressurized and tested separately.

⚠ CAUTION

Risk of process delay. If the clamp below the Non-Target Cell Bag has not been removed after completion of the integrity test, non-target (labeled) cells could not be removed from the separation column during elution. Further retention of non-target cells is then impossible. The separation procedure needs to be repeated. Remove the clamp after completion of the integrity test.

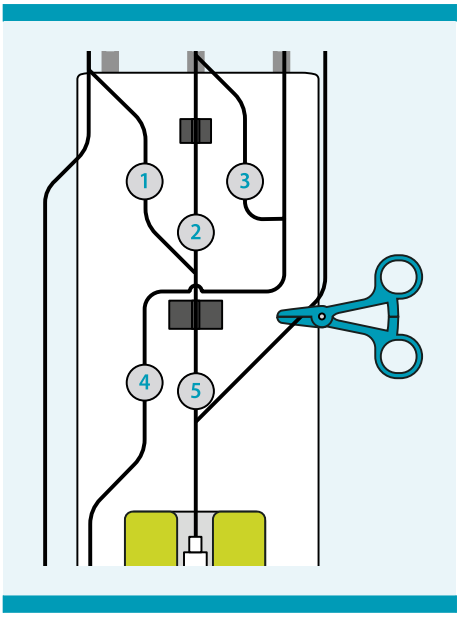


Figure 7.19: Preparation for integrity test

IMPORTANT

The integrity test will only work properly if the tubing is clamped below the Non-Target Cell Bag. Clamp the tubing below the Non-Target Cell Bag, before starting the integrity test. Remove the clamp after the integrity test is completed.

Integrity test for the upper part of the tubing set

1. When the operator performs “Final check of all tubing and attachments” the window indicates Screen 7.63.

2. After performing the “Final check of all tubing and attachments”, **do not** press ENT.

3. To enter the integrity test for the upper part, press 9.

   The window indicates Screen 7.64.

   IMPORTANT
   Clamp the tubing underneath the check valve of the Non-Target Cell Bag.

4. To start the test sequence, press RUN.

   To return to Screen 7.63, press ◄.

5. Once RUN has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window indicates Screen 7.65.

   Overpressure will be created and held for two minutes. During this time the operator should watch all the connections above the upper pump tubing connection.

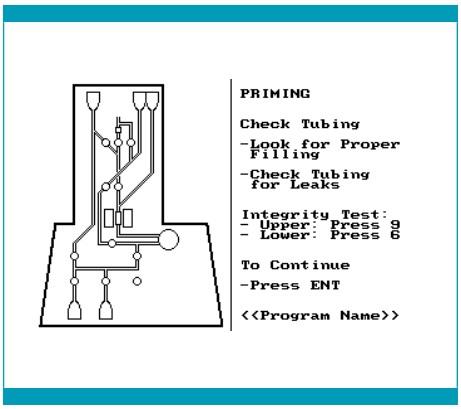
   At each point the test sequence can be finished by pressing ENT.

6. After two minutes the pressure is automatically released, and the window indicates Screen 7.63.

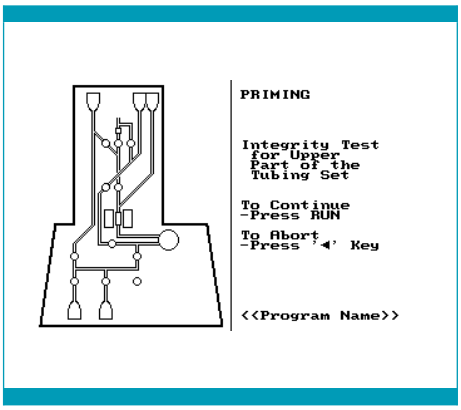
   Check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one.

   Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

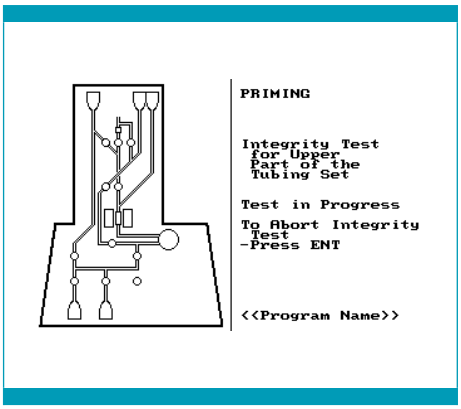
7. If no leakages are observed, continue with the integrity test of the lower part of the tubing set.



Screen 7.63: Start integrity test for upper part



Screen 7.64: Start integrity test for upper part



Screen 7.65: Start integrity test for upper part

Integrity test for the lower part of the tubing set

The window indicates Screen 7.63.

1. To enter the integrity test, press 6.
   Do not press ENT.

   The window indicates Screen 7.66.

2. To start the test sequence, press RUN.

   To return to Screen 7.63, press ◄.

3. Once RUN has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

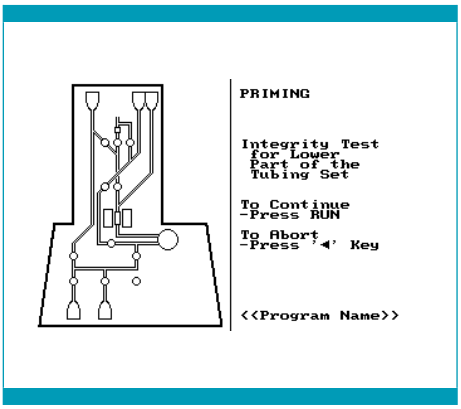
   The window indicates Screen 7.67.

   Overpressure will be created and held for 30 seconds.

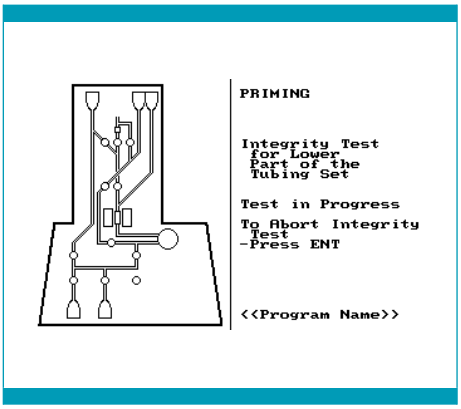
   During this time the operator should watch the lower pump tubing connection and the T-fittings between valves nos. 6, 8, 9, and 10.

   At each point the test sequence can be finished by pressing ENT.

4. After 30 seconds the pressure is automatically released, and the window indicates Screen 7.63.



Screen 7.66: Start integrity test for lower part



Screen 7.67: Integrity test for lower part in process

The operator has to check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one.

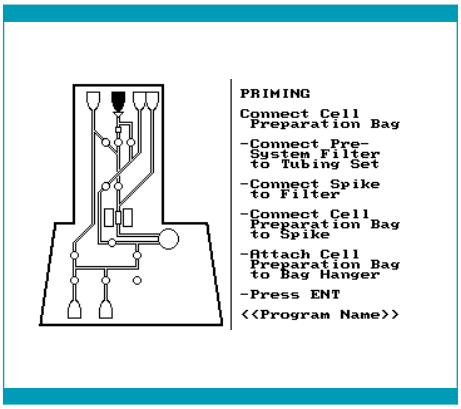
Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

5. **Open the pathway to the Non-Target Cell Bag by removing the clamp underneath the check valve.**

6. If no leakages are observed, the operator can now continue with the next step by pressing ENT.

Connect Cell Preparation Bag

The window indicates Screen 7.68.



Screen 7.68: Connect Cell Preparation Bag

Connect the Cell Preparation Bag (containing the magnetically labeled cells) with the tubing set:

1. Remove the twist-off cap (see Figure 7.12 on page 170) from the tubing set.
2. Remove the cap from the spike of the pre-system filter. Firmly insert the spike of the pre-system filter into the tubing set, ensuring the septum is punctured.
3. Remove the caps from the pre-system filter and the blunt end of the spike connector and connect both part to each other.
4. Remove the other cap from the spike connector and connect the spike to the Cell Preparation Bag ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ensure that the spike has penetrated the bag.
5. Check the connection between the Cell Preparation Bag, spike connector, the pre-system filter and the tubing set to confirm that the connection is secure.
6. Hang the Cell Preparation Bag on the bag hanger (see Figure 7.21).
7. Make sure the bags and tubings attached to the bag hanger are neither stretched nor bent.

   Due to the gravimetric priming and rinsing steps performed during the automated separation it is very important that the bags are leveled correctly. Also, for ensuring the complete processing of sample, it is crucial to place Reapplication and Non-Target Cell Bag higher than Cell Prepration Bag (see Figure 7.20):

   - **Highest position:** buffer bag
   - **Middle position:** Reapplication and Non-Target Cell Bag
   - **Lowest position:** Cell Preparation Bag

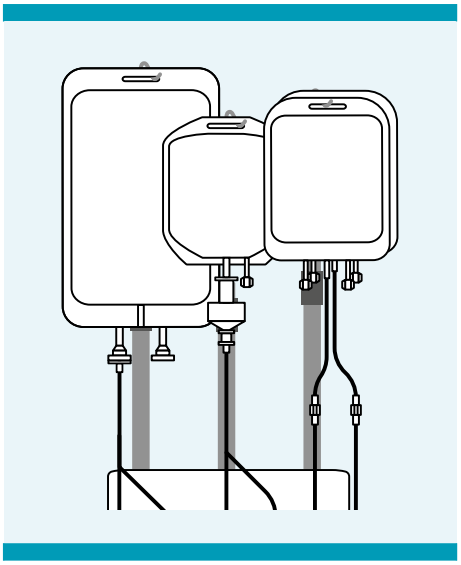


Figure 7.20: Correctly leveled bags

To proceed, press **ENT**.

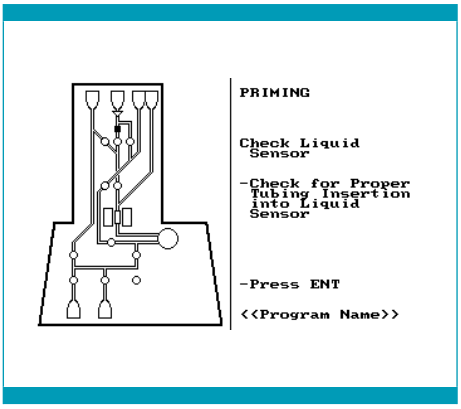
Final check of the liquid sensor

The window indicates Screen 7.69.

1. Check the liquid sensor tubing. Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.
2. Confirm that the unrestricted flow of fluid is possible to each bag.

To proceed, press **ENT**.

▶ **Proceed to STEP 4.**



Screen 7.69: Final check of the liquid sensor

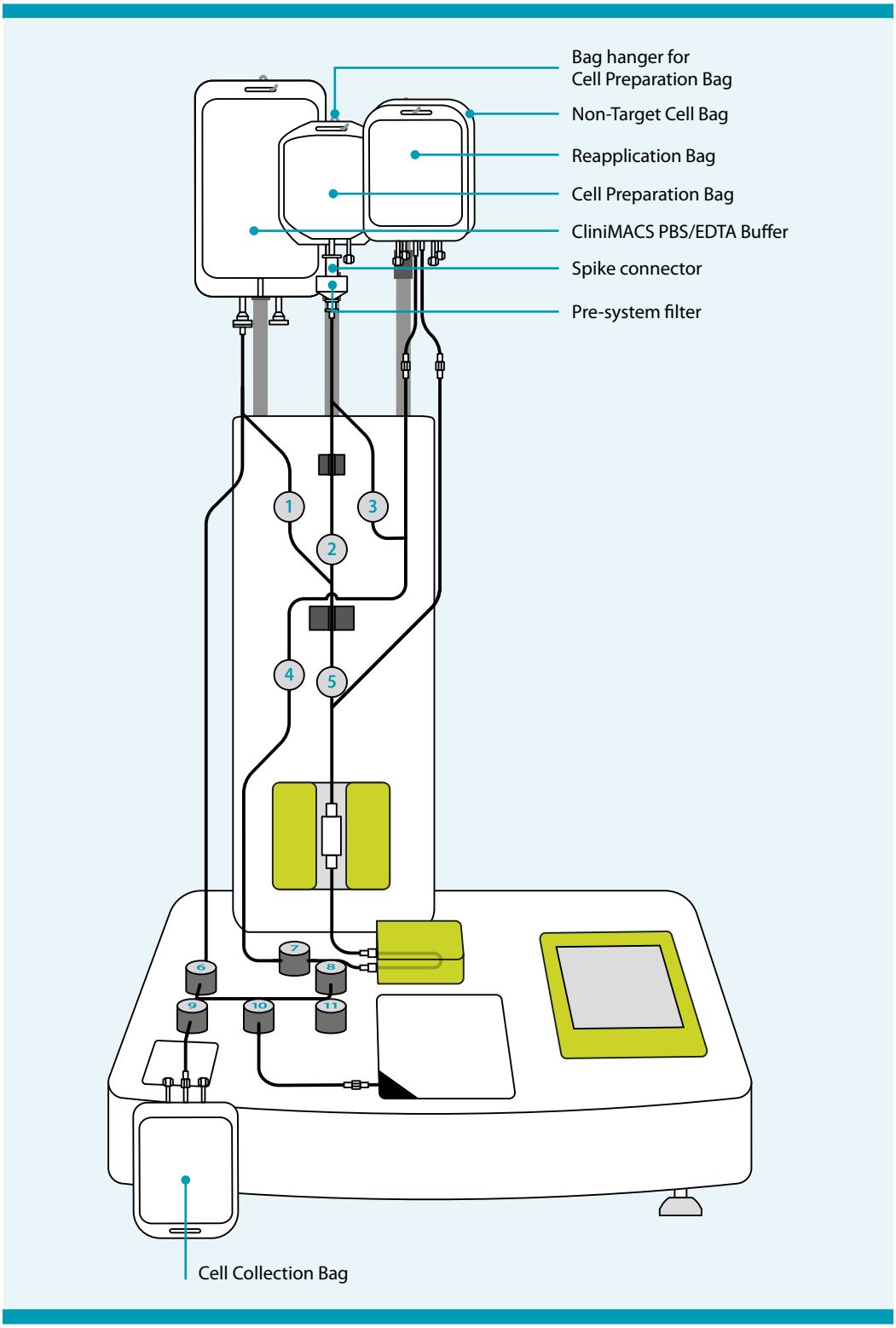
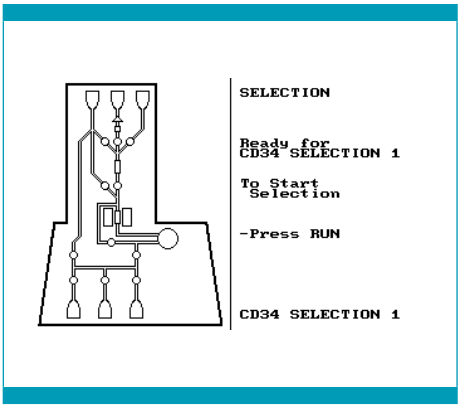


Figure 7.21: CliniMACS Plus Instrument with CliniMACS Depletion Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag

7.4 STEP 4: CliniMACS Plus Separation

7.4.1 CD34 SELECTION 1/2

Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window indicates Screen 7.70.

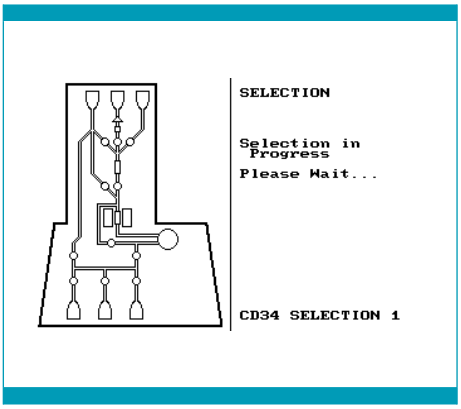


Screen 7.70: Start separation

To proceed, press **RUN**.

Once **RUN** has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 7.71 is indicated to show the status of the separation procedure.



Screen 7.71: Separation in process

The magnet position indicator is shown as two black boxes next to the separation column when the magnet is “ON”, i.e., it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g., Screen 7.70), the magnet is “OFF”, i.e., it has been moved to the rear of the instrument. With the magnet withdrawn, the separation column is outside the magnetic field and is not magnetized any longer.

Note: The screen may show a different program name depending on the chosen separation program (CD34 SELECTION 1 or 2).

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter.

Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set and loads the cell product via the pre-column on the separation column. The magnetically labeled cells (target cells) are retained in the separation column, placed in the magnetic field, while the non-labeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor) the pre-system filter is rinsed twice with buffer.

Column wash I

The pre-column and separation column are washed extensively to remove all non-labeled cells. Wash buffer is collected in the Buffer Waste Bag. When 'Column Wash I' starts, the total remaining time until the end of the separation procedure is shown.

IMPORTANT

At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.

Release of cells I

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied onto the separation column.

Column wash II

Reloading of the cells is followed by a second washing step to remove remaining non-labeled cells. Also all tubings are rinsed several times.

Release of cells II, reloading of cells II, column wash III

The cells are released and reapplied on the separation column for a second time in order to remove any non-labeled cells that unspecifically stick to the column matrix. Afterwards the separation column is washed again.

Release of cells III, reloading of cells III, column wash IV

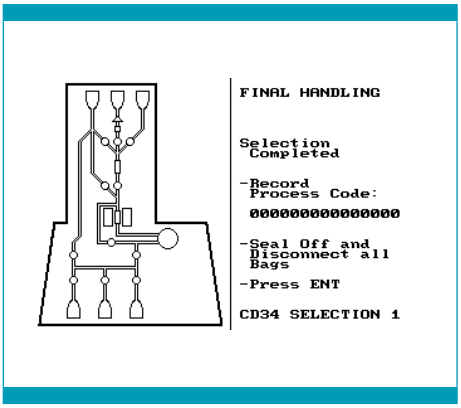
Additionally, the separation program CD34 SELECTION 2 includes a third release and reapplication step.

Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled cells are released from the separation column and collected in the attached Cell Collection Bag.

Disconnect bags and record process code

When the run has been completed, the window indicates Screen 7.72.



Screen 7.72: Disconnect bags and record process code

1. Record the process code.
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.22).

   Make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
3. Weigh the filled Cell Collection Bag. Record the weight.

   Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.
4. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, e.g., 0.5 mL and retain for analysis.
5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag. Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.
6. Disconnect the Buffer Waste Bag in the same way.
7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

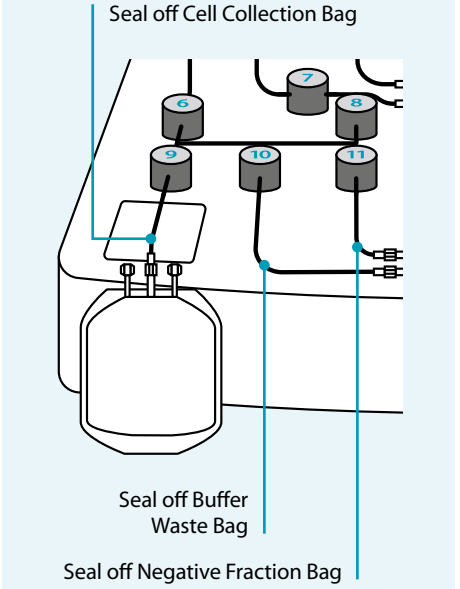


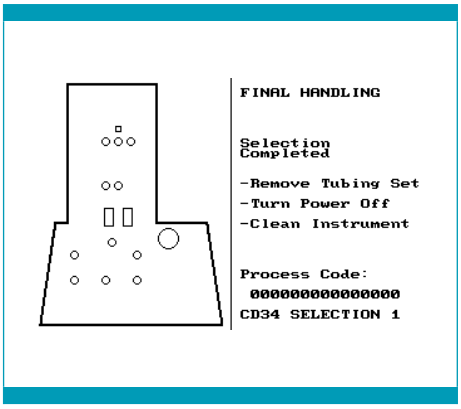
Figure 7.22: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press **RUN**.

Unload tubing set and shutdown

The window indicates Screen 7.73.



Screen 7.73: Unload tubing set and shutdown

1. Remove the tubing set: Beginning with valve nos. 6, 9, 10, and 11, and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2. Switch off the CliniMACS Plus Instrument.
3. Clean the instrument according to cleaning instructions, see section "Cleaning and disinfection" in the CliniMACS Plus Instrument User Manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

IMPORTANT

- *The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.*
- *If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.*

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.2 ENRICHMENT 1.1

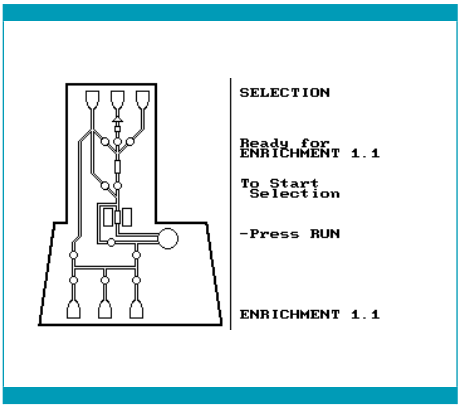
Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window indicates Screen 7.74.

To proceed, press **RUN**.

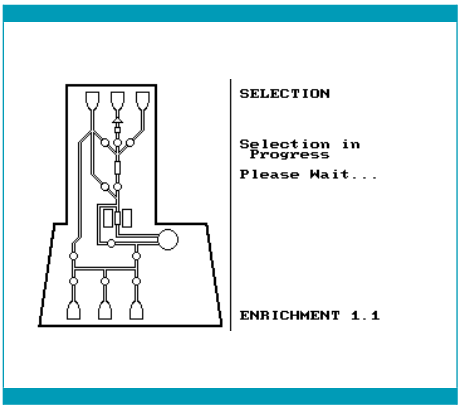
Once **RUN** has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 7.75 is indicated to show the status of the separation procedure.

The magnet position indicator is indicated as two black boxes next to the separation column when the magnet is "ON", i.e., it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g., Screen 7.74), the magnet is "OFF", i.e., it has been moved to the rear of the instrument. With the magnet withdrawn, the separation column is outside the magnetic field and is not magnetized.



Screen 7.74: Start separation



Screen 7.75: Separation in process

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set and loads the cell product via the pre-column on the separation column. The magnetically labeled cells (target cells) are retained in the separation column, placed in the magnetic field, while the non-labeled cells (non-target cells) are passed through and are collected in the Negative Fraction Bag.

IMPORTANT

At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.

Note:

- If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in suitable portions ("staged loading").
- The loading will be stopped when the capacity of the separation column is reached, and the separation program will proceed to the next step of the separation. After the last step (final elution of the cells) has been completed, the next portion of the sample will be loaded onto the column and processed accordingly.

Column wash I

The pre-column and separation column are washed extensively to remove all non-labeled cells. Wash buffer is collected in the Buffer Waste Bag.

Release of cells

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but remain within an internal tubing cycle.

Reloading of cells

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied onto the separation column.

Column wash II

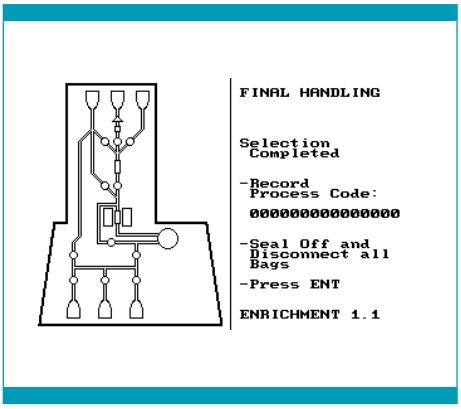
Reloading of the cells is followed by a second column washing sequence to remove remaining non-labeled cells. Also all tubing are rinsed several times.

Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled cells (target cells) are released from the separation column and collected in the Cell Collection Bag.

Disconnect bags and record process code

When the run has been completed, the window indicates Screen 7.76.



Screen 7.76: Disconnect bags and record process code

1. Record the process code.
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.23).

   Make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
3. Weigh the filled Cell Collection Bag. Record the weight.

   Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.
4. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, e.g., 0.5 mL and retain for analysis.
5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag. Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.
6. Disconnect the Buffer Waste Bag in the same way.
7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

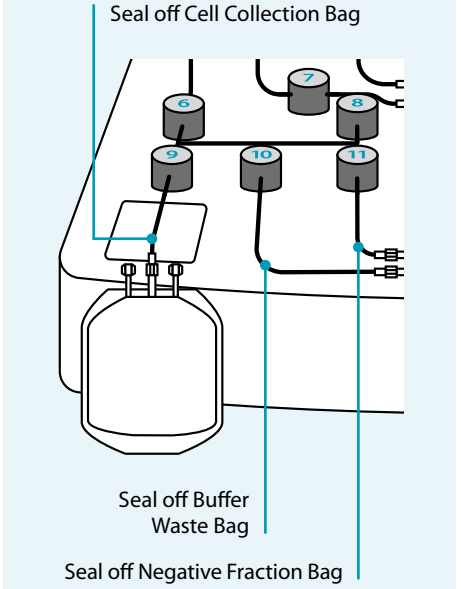


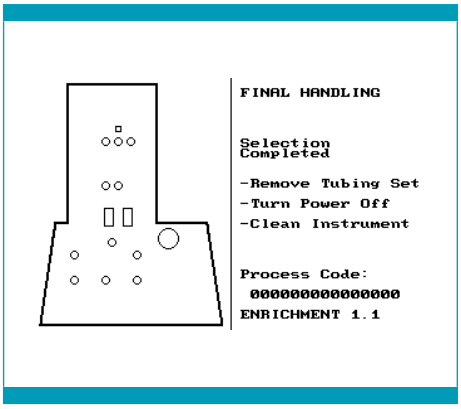
Figure 7.23: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press **ENT**.

Unload tubing set and shutdown

The window indicates Screen 7.77.



Screen 7.77: Unload tubing set and shutdown

1. Remove the tubing set: Beginning with valve nos. 6, 9, 10, and 11, and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2. Switch off the CliniMACS Plus Instrument.
3. Clean the instrument according to cleaning instructions, see section "Cleaning and disinfection" in the CliniMACS Plus Instrument User Manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

IMPORTANT

- *The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.*
- *If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.*

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity (depletion efficiency) and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.3 ENRICHMENT 3.2

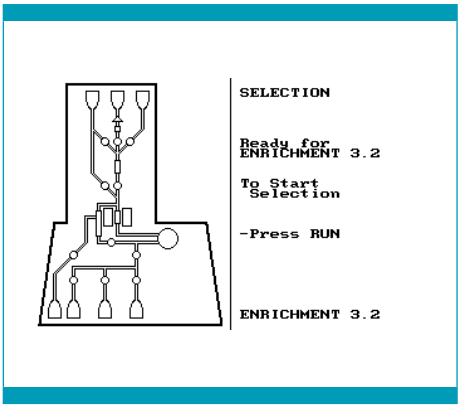
Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window indicates Screen 7.78.

To proceed, press **RUN**.

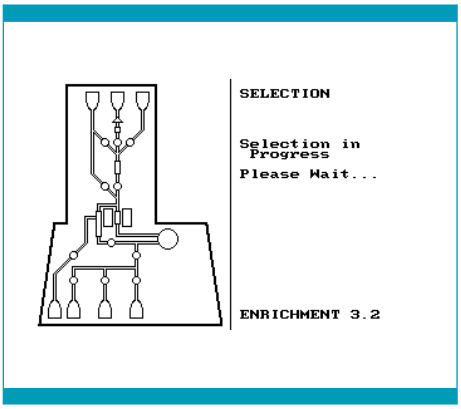
Once **RUN** has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 7.79 is indicated to show the status of the separation procedure.

The magnet position indicator is indicated as two black boxes next to the separation column when the magnet is "ON", i.e., it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g., Screen 7.78), the magnet is "OFF", i.e., it has been moved to the rear of the instrument. With the magnet withdrawn, the separation column is outside the magnetic field and is not magnetized.



Screen 7.78: Start separation



Screen 7.79: Separation in process

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set and loads the cell product via the pre-column on the separation column. The magnetically labeled cells (target cells) are retained in the separation column, placed in the magnetic field, while the non-labeled cells (non-target cells) are passed through and are collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor), the pre-system filter is rinsed twice with buffer.

IMPORTANT

At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.

Column wash I

The pre-column and separation column are washed extensively to remove all non-labeled cells. Wash buffer is collected in the Buffer Waste Bag.

Release of cells I

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied onto the separation column.

Column wash II

The reloading of the cells is followed by a second washing step to remove remaining non-labeled cells. Also, all tubing are rinsed several times.

Release of cells II, reloading of cells II, column wash III

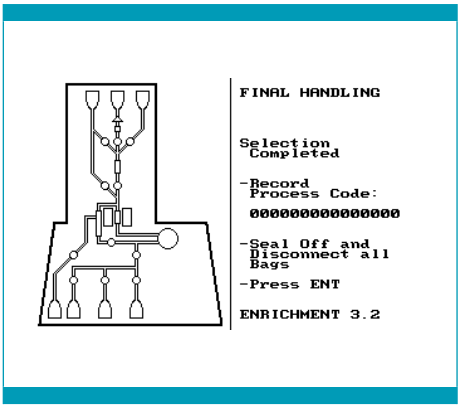
The cells are released and reapplied onto the separation column for a second time in order to remove any non-labeled cells sticking unspecifically to the column matrix. Afterwards the separation column is washed again.

Final elution of cells

The magnet is moved into the "OFF" position and the magnetically labeled cells (target cells) are released and collected in the Cell Collection Bag.

Disconnect bags and record process code

When the run has been completed, the window indicates Screen 7.80.



Screen 7.80: Disconnect bags and record process code

1. Record the process code.
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.24).

   Make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
3. Weigh the filled Cell Collection Bag. Record the weight.

   Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.
4. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, e.g., 0.5 mL and retain for analysis.
5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag. Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.
6. Disconnect the Buffer Waste Bag in the same way.
7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

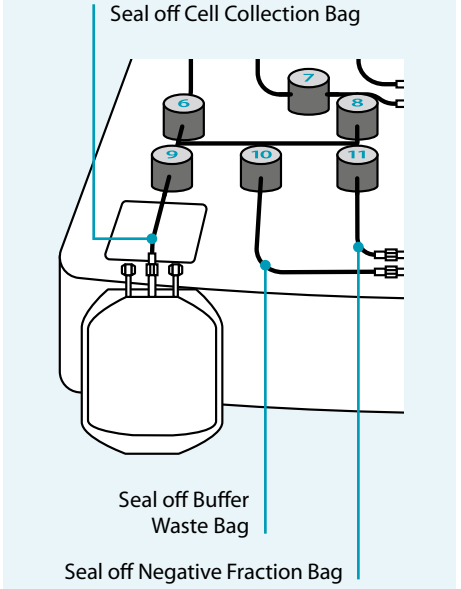


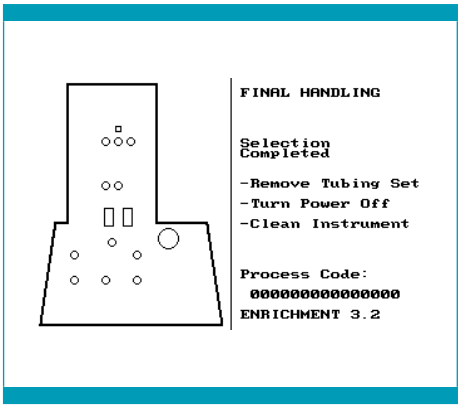
Figure 7.24: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press **ENT**.

Unload tubing set and shutdown

The window indicates Screen 7.81.



Screen 7.81: Unload tubing set and shutdown

1. Remove the tubing set: Beginning with valve nos. 6, 9, 10, and 11, and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2. Switch off the CliniMACS Plus Instrument.
3. Clean the instrument according to cleaning instructions, see section "Cleaning and disinfection" in the CliniMACS Plus Instrument User Manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

IMPORTANT

- *The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.*
- *If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.*

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.4 DEPLETION 2.1

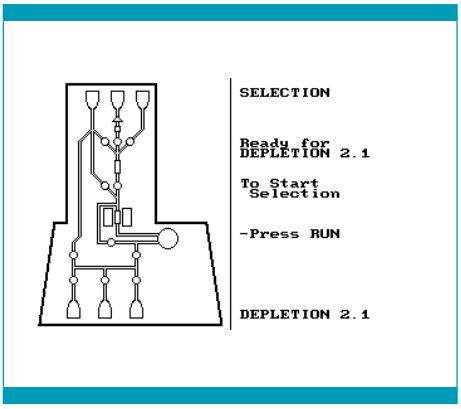
Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window indicates Screen 7.82.

To proceed, press **RUN**.

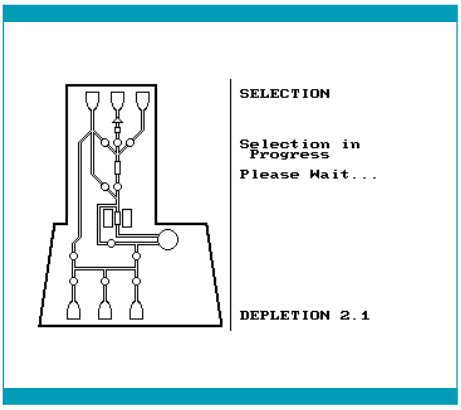
Once **RUN** has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 7.83 is indicated to show the status of the separation procedure.

The magnet position indicator is indicated as two black boxes next to the separation column when the magnet is "ON", i.e., it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g., Screen 7.82), the magnet is "OFF", i.e., it has been moved to the rear of the instrument. With the magnet withdrawn, the separation column is outside the magnetic field and is not magnetized.



Screen 7.82: Start separation



Screen 7.83: Separation in process

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells (non-target cells) are retained in the separation column, placed in the magnetic field, while the non-labeled cells (target cells) are passed through and collected in the Cell Collection Bag.

IMPORTANT

At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.

Rinsing pre-system filter

After the cell sample has been completely loaded onto the tubing set, the pre-system filter is rinsed twice with buffer to reduce cell loss in the filter.

Note:

- If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").
- The loading will be stopped when the capacity of the separation column is reached and the separation program will proceed to the next step of the separation. After the last step (final elution of the cells) has been completed, the next portion of the sample will be loaded onto the column.

Column wash

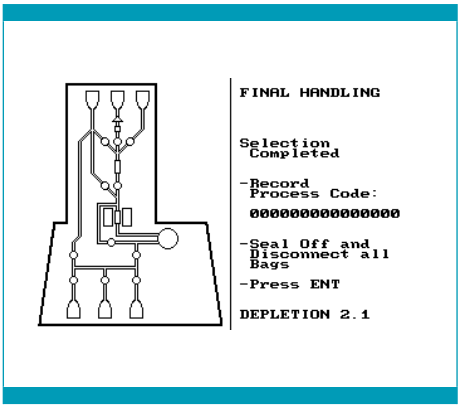
The pre-column and separation column are washed extensively to remove all non-labeled cells. Wash buffer is collected in the Buffer Waste Bag.

Final elution of the cells

The magnet is moved into the "OFF" position. At first the magnetically labeled cells are eluted to the Priming Waste Bag. Only after the last loading stage has been finished, all magnetically labeled cells (non-target cells) are released from the separation column and collected in the Negative Fraction Bag to reduce carryover with labeled cells in the pump tubing.

Disconnect bags and record process code

When the run has been completed, the window indicates Screen 7.84.



Screen 7.84: Disconnect bags and record process code

1. Record the process code.
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.25).

   Make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
3. Weigh the filled Cell Collection Bag. Record the weight.

   Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.
4. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, e.g., 0.5 mL and retain for analysis.
5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag. Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.
6. Disconnect the Buffer Waste Bag in the same way.
7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

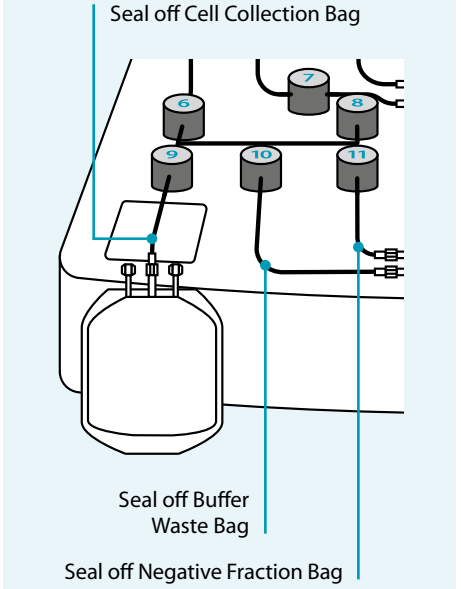


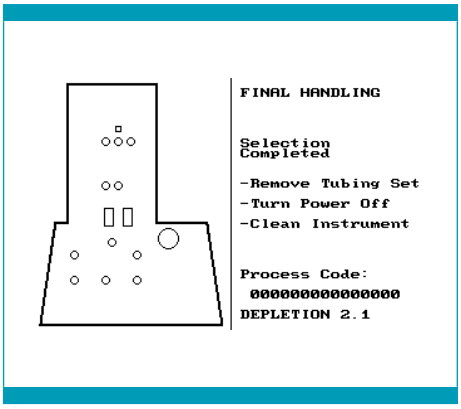
Figure 7.25: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press **ENT**.

Unload tubing set and shutdown

The window indicates Screen 7.85.



Screen 7.85: Unload tubing set and shutdown

1. Remove the tubing set: Beginning with valve nos. 6, 9, and 10 and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the column from the column holder. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2. Switch off the CliniMACS Plus Instrument.
3. Clean the instrument according to cleaning instructions, see section "Cleaning and disinfection" in the CliniMACS Plus Instrument User Manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

IMPORTANT

- *The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.*
- *If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.*

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- depletion efficiency

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.5 DEPLETION 3.1

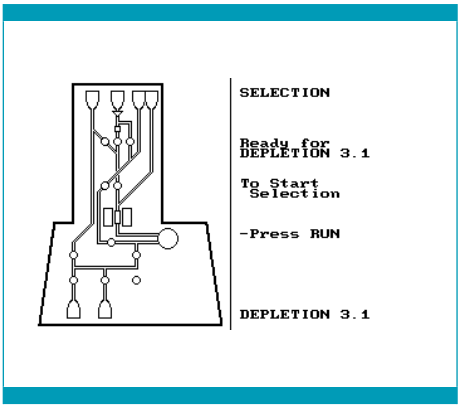
Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window indicates Screen 7.86.

To proceed, press **RUN**.

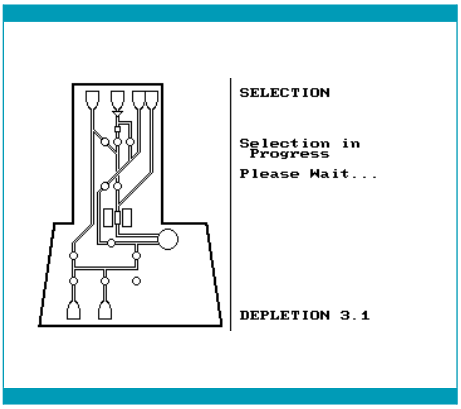
Once **RUN** has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 7.87 is indicated to show the status of the separation procedure.

The magnet position indicator is indicated as two black boxes next to the separation column when the magnet is "ON", i.e., it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g., Screen 7.86), the magnet is "OFF", i.e., it has been moved to the rear of the instrument. With the magnet withdrawn, the separation column is outside the magnetic field and is not magnetized



Screen 7.86: Start separation



Screen 7.87: Separation in process

Separation procedure

Depletion 3.1 is a two-step depletion program, i.e. the cells are applied onto the separation column twice.

In the first part of the process the bulk of labeled cells is depleted (Staged Cell Loading [Bulk Loading Stage]). In the second part of the process the few remaining labeled cells are depleted (Sensitive Sample Loading [Sensitive Loading Stage]).

IMPORTANT

At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.

Note: If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading"). Between each loading stage, the separation column is washed to remove all labeled cells. The labeled cells will be eluted and held in the Non-Target Cell Bag. The wash buffer is collected in the Reapplication Bag.

Staged cell loading (Bulk Loading Stage)

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells (non-target cells) are retained in the separation column, placed in the magnetic field, while the non-labeled cells (target cells) are passed through and collected in the Reapplication Bag.

Sensitive sample loading (Sensitive Loading Stage)

For further depletion the cells in the Reapplication Bag are reloaded to the separation column at a lower speed. While the few remaining labeled cells are now retained on the column, the non-labeled cells (target cells) flow through the magnetic field and are collected in the Cell Collection Bag.

Rinsing pre-system filter and Reapplication Bag

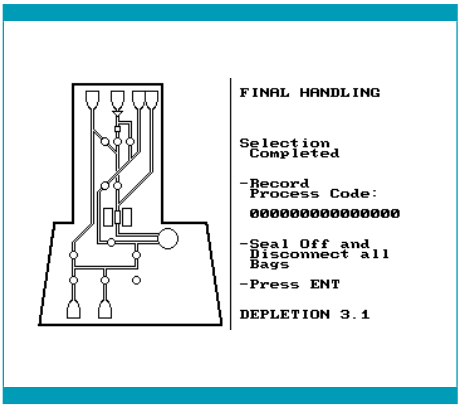
After the cell sample has completed both, bulk and sensitive depletion, the pre-system filter and the Reapplication Bag are rinsed with buffer twice to reduce cell loss in the system.

Removal of non-target cells

The magnet is moved into the “OFF” position. The magnetically labeled cells (non-target cells) are released from the separation column and collected into the Non-Target Cell Bag after each loading stage.

Disconnect bags and record process code

When the run has been completed, the window indicates Screen 7.88.



Screen 7.88: Disconnect bags and record process code

1. Record the process code.
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.26).

   Make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
3. Weigh the filled Cell Collection Bag. Record the weight.

   Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.
4. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, e.g., 0.5 mL and retain for analysis.
5. Using the heat sealer, seal off the tubing above the luer lock of the Buffer Waste Bag. Make three hermetic seals in the tubing. Sever the center seal to disconnect the Buffer Waste Bag.
6. Disconnect the Non-Target Cell Bag in the same way.
7. Remove the Non-Target Cell Bag and the Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

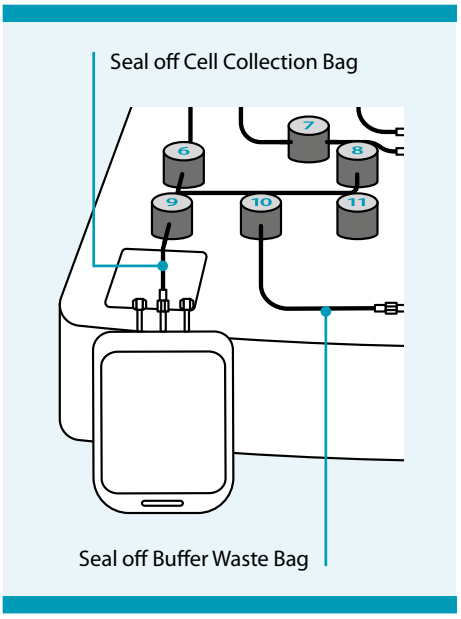


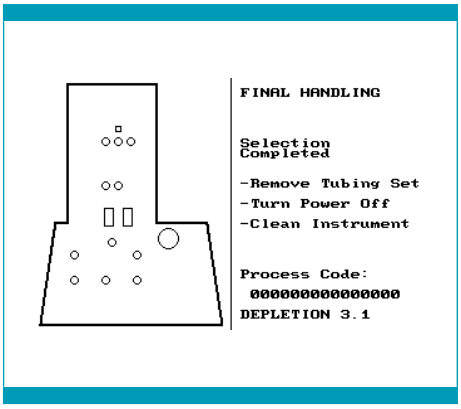
Figure 7.26: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press **ENT**.

Unload tubing set and shutdown

The window indicates Screen 7.89.



Screen 7.89: Unload tubing set and shutdown

1. Remove the tubing set: Beginning with valve nos. 6, 9, and 10 and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the column from the column holder. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2 Switch off the CliniMACS Plus Instrument.
3. Clean the instrument according to cleaning instructions, see section "Cleaning and disinfection" in the CliniMACS Plus Instrument User Manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

IMPORTANT

- *The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.*
- *If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.*

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- depletion efficiency

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

8 Troubleshooting

This chapter is intended as a reference to provide information about possible unexpected events that might occur and to suggest appropriate corrective action. For information not covered in the following chapter, contact Miltenyi Biotec Technical Support as soon as possible.

Note: The order of this chapter follows the actual sequence of a separation.

8.1 Miltenyi Biotec Technical Support

In any case of instrument malfunction or process failure, which cannot be corrected by the operator (error messages), contact the Miltenyi Biotec Technical Support team:

+49 2204 8306-3803
technicalsupport@miltenyi.com

Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

8.2 Preparation of the leukapheresis product

The sample received is diluted

Ideally, magnetic labeling is performed in diluted leukapheresis product. Adjust the leukapheresis product to a final dilution of approximately 1:3. If the sample received is more diluted than 1:3, or if the operator does not exactly know the concentration of plasma in the sample, add immunoglobulin to the sample prior to the addition of the reagent (recommended concentration of immunoglobulin in the labeling volume: 1.5 mg/mL). It is important to have a certain amount of immunoglobulin in the sample during the labeling in order to minimize non-specific binding of the reagent.

The number of target cells is low in the leukapheresis product

The mobilization of stem cells was insufficient. Check analysis of the leukapheresis product.

■ Poor viability of cells in the leukapheresis product

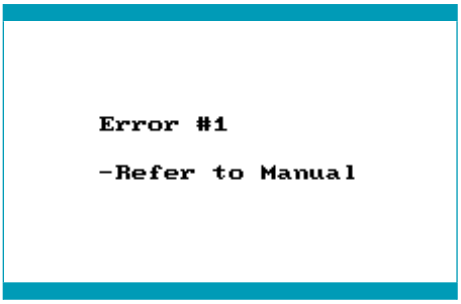
The leukapheresis product may have been harvested, stored or transported inappropriately. To ensure better sample quality, the preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis product with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

8.3 CliniMACS Plus Instrument and CliniMACS Tubing Sets

8.3.1 Error messages

There are a number of possible instrument or software malfunctions. These are marked as such and will be indicated on the screen. They refer to internal errors that cannot be corrected by the operator. Record the error number and contact Miltenyi Biotec Technical Support.

One possible error message is shown in Screen 8.1.



Screen 8.1: Error message no. 1

Other than error messages, malfunctions that can be corrected by the operator are marked “Warning messages” (see section 8.4.1).

8.3.2 Loading and priming of the tubing set

■ Valve does not open when operator is instructed to insert tubing into a particular valve

The valves are designed to work properly once the tubing has been inserted. Press the valve manually to open it. Watch the valve carefully during the valve exercise sequence. If the valve does not depress during the valve exercise sequence, see section "Valve does not depress during valve exercise sequence".

■ Valve does not depress during valve exercise sequence

Confirm that tubing is correctly inserted. Check whether valves have been cleaned thoroughly. Any valve that has been contaminated by fluid has to be exchanged. Contact Miltenyi Biotec Technical Support.

- Buffer is leaking from tubing set during priming

  Tubing set is defective. Turn off the CliniMACS Plus Instrument and restart priming with a new tubing set installed and sufficient new buffer.

- Excessive air occurs in tubing set after priming

  Buffer bag is not properly spiked. Use a new tubing set and sufficient new buffer and restart the CliniMACS Plus Separation. Confirm that the septum of the buffer bag is properly punctured.

- Unexpected volume of buffer in bags after priming. After priming, liquid should only be in the Priming Waste Bag and Buffer Waste Bag

  Tubing set is not mounted correctly. Liquid can leak behind the valves if the tubing set is not installed correctly or the valves are not functioning properly. Remove the tubing set and replace it with a new one. Restart the priming procedure with sufficient new buffer. Poor performance of the CliniMACS Plus Separation may result if the tubing set is not inserted properly.

- Pump motor stalls during priming

  Pump tubing has not been inserted correctly. Press STOP to interrupt the priming and turn the power "OFF" and then "ON" again. Clamp the buffer line with a locking forceps during the installation procedure, correct the position of the pump tubing and remove the locking forceps before restarting the priming sequence.

8.4 Automated cell separation

8.4.1 Warning messages

Unlike error messages (see section 8.3.1), warning messages are indicated on the screen when the internal control system of the CliniMACS Plus Instrument recognizes a malfunction which can be corrected by the operator. Usually, a warning message appears in combination with a sound ("beep"). If a warning message appears during the CliniMACS Plus Separation, follow the instructions on the screen to proceed with the cell separation. Generally speaking, warning messages appear when the STOP is pressed, when the pump door is opened, when the pump stalls or when the liquid sensor detects an error.

Since different kinds of unexpected events can occur during different separation programs, the following section is subdivided accordingly.

8.4.2 Unexpected events: All separation programs

Error detected by liquid sensor

Warning message no. 1 will appear during the starting phase of the cell loading process if the liquid sensor is not able to detect liquid in the tubing. As the pre-system filter is rinsed with buffer prior to the cell loading, there must be liquid in the tubing at this point.

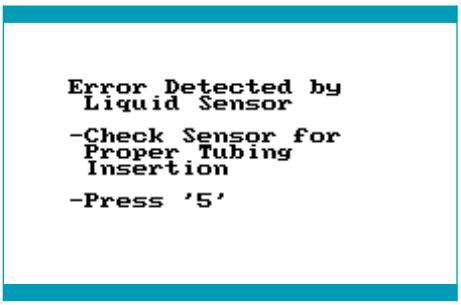


Figure 8.1: Warning message no. 1

Check the following points:

1. Has the tubing been inserted correctly? Into the liquid sensor? If not, do so.
2. Is the tubing filled with buffer? If not, see point 3.
3. If the tubing is not filled with buffer, inspect the tubing for kinks blocking the buffer flow upwards into the pre-system filter and Cell Preparation Bag. Adjust the position of the tubing set. If necessary, raise or lower the bag hangers using the bag hanger clamps. Adjust the position of the tubing in the valves. To alter the position of the tubing, open the valve by manually pressing the button. Confirm that the tubing is not kinked, twisted or taut.
4. Is the Cell Preparation Bag spiked properly? Confirm the pre-system filter spike has penetrated the septum of the Cell Preparation Bag port.
5. If the tubing set has not been completely filled with buffer: Has the buffer spike of the tubing set penetrated the buffer bag? For correction see "Excessive air occurs in tubing set after priming" in section 8.3.2.

After the corrective action, continue with the separation in progress and press 5. If warning message no. 1 appears again after each of the possible causes listed above have been ruled out, the liquid sensor may be defect. Contact Miltenyi Biotec Technical Support.

Cells move to wrong part of tubing set. Liquid is leaking past valve(s)

- Tubing set has not been properly inserted. If the instrument run is ongoing, press STOP and clamp the line with a locking forceps. Adjust the tubing by first depressing the appropriate valve. Remove the locking forceps and press RUN to resume separation. The cell separation will be aborted if RUN is not pressed within 600 seconds.
- Valve is not functioning properly. Press STOP. Clamp the line with a locking forceps. Depress the valve manually several times to unstick the stuck valve. Remove the locking forceps and press RUN to continue. The cell separation will be aborted if RUN is not pressed within 600 seconds. If the user is unable to unstick the valve, contact Miltenyi Biotec Technical Support.

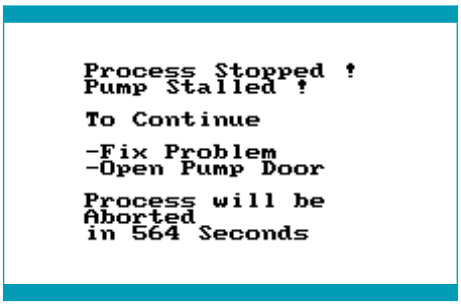
- Wrong software program used. Check display for name of program currently used. Abort run by pressing the STOP and immediately contact Miltenyi Biotec Technical Support.

Magnet does not move

Magnet drive does not work. A magnet drive failure has occurred. An error message will be indicated (see section 8.3.1). Record the number of the error message and contact Miltenyi Biotec Technical Support.

Pump motor stalls during cell separation

Pump tubing has not been inserted correctly, so the pump might be unable to rotate. In this case warning message no. 2 will appear on the screen window. The operator then has 600 seconds to correct the position of the pump tubing.



Screen 8.2: Warning message no. 2

1. Carefully remove the pump tubing from the pump.
2. Confirm that the pump tubing has not been damaged by the incorrect insertion. If the pump tubing is leaking, clamp the tubing above and below the separation column to save the cells retained on the column and contact Miltenyi Biotec Technical Support.
3. If the pump tubing has not been damaged, it can be reinserted into the pump housing.
4. Press RUN to restart the separation within 600 seconds or the separation will be aborted.

If the pump tubing is flat and collapsed, this hints to a clogged column. In this case, contact Miltenyi Biotec Technical Support.

Sample loading does not stop although the Cell Preparation Bag and the pre-system filter are empty

Liquid sensor is not working properly, e.g., because the surface of the tubing in the liquid sensor is wet. Press STOP to interrupt the separation. Remove tubing from liquid sensor. Dry the tubing and the contact area using a paper towel or absorbent material. Replace the tubing in the liquid sensor and press RUN. Do not interrupt the sequence for more than 600 seconds or the cell separation will be terminated.

If it is not possible to activate the liquid sensor in this way, press STOP, then 2 and confirm with ENT to skip sample loading and to continue the separation.

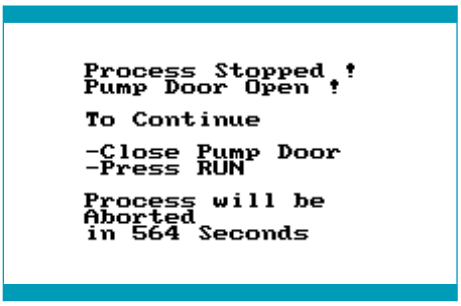
8.4.3 Unexpected events: Enrichment programs only

Run is aborted before completion of cell separation program

- Pump door opened: For safety reasons, the separation in progress will automatically stop during the instrument run if the pump door is opened. Message no. 5 will appear on the screen window.

  Close the pump door and press **RUN**.

  If, as in this case, **RUN** is not pressed within 600 seconds, the separation will be aborted, and the cell separation will not be completed. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.



Screen 8.3: Warning message no. 5

- Power failure: Power failure results in the termination of the CliniMACS Plus Separation. The cell separation will not be completed once the power supply has been restored. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

8.4.4 Unexpected events: CD34 SELECTION 1/2

Loading stopped before complete sample has been loaded onto the columns

Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles activating the liquid sensor. Therefore, the separation process has been continued with the column washes before all of the sample could be loaded. It is not possible to restart the sample loading once the loading sequence has stopped.

Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

Pump tubing collapsed, and/or excessive air appeared in tubing set below pre-column during cell loading

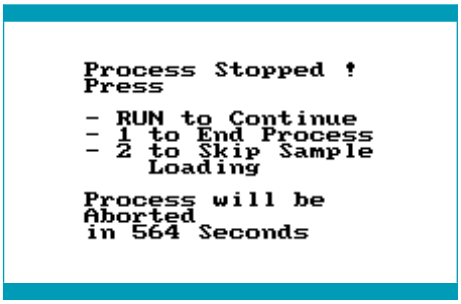
Pre-column is clogged due to large amount of cell debris in the Cell Preparation Bag. It is necessary to skip the loading of the remaining sample manually and to continue the separation with the cells that have already been loaded onto the system.

1. Press STOP to interrupt cell loading. Warning message no. 3 will appear.
2. Press 2 to skip the cell loading process.

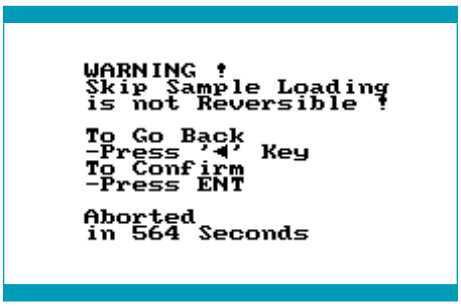
   Warning message no. 4 will appear and will give the operator the opportunity to confirm or amend the decision because skipping of sample loading is not reversible.
3. Press ENT and the separation program will stop the sample loading and continue with column washes.

Clamp the tubing below the pre-system filter to prevent cells from leaking out of the Cell Preparation Bag into the tubing set. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag.

Immediately perform a second separation with a new tubing set and sufficient new buffer.



Screen 8.4: Warning message no. 3



Screen 8.5: Warning message no. 4

8.4.5 Unexpected events: ENRICHMENT 1.1

- Loading stopped before complete sample has been loaded onto the columns

  Pre-system filter is clogged due to large amount of cell debris or due to incomplete filing of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles. These have activated the liquid sensor and the separation process has been continued directly with the final stage of sample loading. A part of the sample may remain in the Cell Preparation Bag after separation is finished.

  Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss.

  Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag and enter actual sample parameters during set-up of the instrument (STEP 2). After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

- Wrong sample parameter values were entered

  The sample loading volume entered was lower than the actual volume in the attached Cell Preparation Bag. It is not possible to restart the sample loading once it has stopped.

  Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss.

  Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag and enter actual sample parameters during set-up of the instrument (STEP 2). Process remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer.

- Pump motor stalls during elution of target cells into Cell Collection Bag

  The locking forceps next to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked.

  Press **STOP** and open the locking forceps next to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press **RUN** to restart the elution sequence within 600 seconds.

8.4.6 Unexpected events: ENRICHMENT 3.2

- Loading stopped before complete sample has been loaded onto the columns

  Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles. These have activated the liquid sensor and the separation process has been continued with the column washes before all of the sample has been loaded. It is not possible to restart the sample loading once the loading sequence has stopped.

  Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss.

  After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

- Pump motor stalls during elution of target cells into Cell Collection Bag

  The locking forceps next to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked.

  Press **STOP** and open the locking forceps next to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press **RUN** to restart the elution sequence within 600 seconds.

8.4.7 Emergency program (to be used with enrichment programs only)

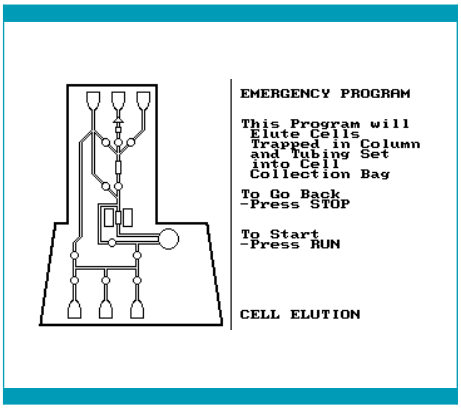
If, for any reason, a run has irreversibly terminated prior to the target cells being eluted from the separation column, the emergency program can be run to elute the cells from the separation column. This program has been designed only for use with all enrichment programs together with either a CliniMACS Tubing Set (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01).

The Emergency Program must not be used with any depletion programs.

Note: The emergency program will elute approximately 75 mL of fluid. Confirm that a suitable Cell Collection Bag is attached to the tubing set.

1. To confirm that the separation column is not magnetized, turn the instrument off, wait 5 seconds, then turn the instrument on again. The magnet will be withdrawn from the magnetic separation unit (see section "Description" in the CliniMACS Plus Instrument User Manual). Check this by holding a small magnetizable item to the magnetic separation unit. If the magnet has not been withdrawn, or if there is an ongoing power failure, contact Miltenyi Biotec Technical Support.
2. Wait until the main screen appears in the window (see section "Installation" in the CliniMACS Plus Instrument User Manual).

   To call up the emergency program, press **4**. The window indicates Screen 8.6.



Screen 8.6: Emergency program

3. To continue with the elution of the trapped cells, press **RUN**.

   Note: To leave the emergency program without starting the elution of the trapped cells, press **STOP**.

4. Transfer the eluted cells collected in the Cell Collection Bag to a new 600 mL transfer bag. Eventually pool the remaining cells in the Cell Preparation Bag with the cells eluted by the emergency program. Start a new separation procedure using a new tubing set and sufficient new buffer.

8.4.8 Unexpected events: DEPLETION 2.1

Target cells do not reach the Cell Collection Bag

Pump is unable to load the product from the Cell Preparation Bag because the clamp next to the Cell Collection Bag is not open during the cell loading sequence. Press **STOP** and open clamp next to Cell Collection Bag. Continue the depletion process by pressing **RUN**.

Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. If a part of the product remains in the Cell Preparation Bag after depletion procedure has been finished, process the remaining cells with a new tubing set, a new pre system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2) and start the new depletion.

Loading stopped before complete sample has been loaded onto the columns

Air bubbles from the sample and/or pre-system filter activated the liquid sensor before all of the sample had been loaded. Abort the current run and check total cell number and depletion efficiency of the cells in the Cell Collection Bag. If necessary, process the remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2) and start the new depletion.

8.4.9 Unexpected events: DEPLETION 3.1

Loading stopped during Staged Cell Loading, before complete sample has been loaded onto the columns

- Pre-system filter inserted wrong way around. Therefore, the drip chamber function is not available and air bubbles may pass the liquid sensor directly causing the termination of the sample loading before the complete sample has been applied. The instrument continues the depletion process by directly beginning the next step. It is not possible to restart the sample loading once it has stopped.
- Pre-system filter is clogged due to large amount of cell debris or due to incomplete filing of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles. These have activated the liquid sensor and the separation process has been continued directly with the final stage of sample loading. A part of the sample may remain in the Cell Preparation Bag after separation is finished.

Abort the current run and pool the contents of the Reapplication Bag and the Cell Preparation Bag. Process the remaining cells with a new tubing set, a new pre-system filter and sufficient new buffer.

Loading stopped during Sensitive Sample Loading (Sensitive Loading Stage)

The Reapplication Bag is hanging lower than the Cell Preparation Bag. During sensitive loading step, Cell Preparation Bag runs empty before Reapplication Bag volume is completely loaded. It is therefore possible that an air bubble activates the liquid sensor before the sample is completely loaded from the Reapplication Bag after the depletion procedure is finished. It is not possible to restart sample loading once it has stopped.

Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. Process remaining sample with a new tubing set, pre-system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2). Consider the obligatory need of three large bag hangers for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see section 7.3.3).

Target cells (non-labeled cells) do not reach Reapplication Bag during Staged Cell Loading Bulk (Loading Stage)

Wrong tubing is inserted in valve no. 3 (right branch of Reapplication Bag tubing instead of left branch (Y-fitting) of tubing). Press **STOP** and remove wrong tubing from valve no. 3. Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no. 3 as described in the installation instructions. Press **RUN** to continue with the depletion procedure within 600 seconds.

Allow the CliniMACS Plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. Check depletion efficiency of the target fraction in the Cell Collection Bag. If depletion efficiency is not sufficient, repeat depletion procedure with a new tubing set, a new pre-system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameter values during set-up of the instrument (STEP 2) and start the new depletion.

Cells flow into buffer bag during gravimetric rinsing steps

Buffer bag is hanging too low (lower than Cell Preparation Bag). Press **STOP** and adjust buffer bag hanger to correct position. Confirm that three large bag hangers are installed for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see section 7.3.3).

- If cells have not reached the buffer bag, it is sufficient to manually open valves nos. 1 and 2 to allow short backflushing of cells into Cell Preparation Bag. After the buffer bag tube is clear again, close valves nos. 1 and 2 and press **RUN** to continue with the depletion procedure within 600 seconds.
- If cells have already reached the buffer bag, exchange buffer bag, manually open valves nos. 1 and 2 for a short flushing of the buffer bag tube and press **RUN** to continue with the depletion procedure within 600 seconds. Process

the remaining sample in the buffer bag if necessary (if volume exceeds 300 mL, transfer the sample into a centrifugable bag for volume reduction first) using a new tubing set, a new pre-system filter, and sufficient new buffer.

■ Pump motor stalls during first elution of labeled cells into Non-Target Cell Bag

- Locking forceps has not been removed after integrity test. Press **STOP** and remove locking forceps. Open pump door and check whether pump tubing is correctly inserted. Close pump door and press **RUN** to restart elution sequence within 600 seconds.
- Wrong tubing is inserted in valve no. 3 (Non-Target Cell Bag tubing instead of left branch (Y-fitting) of Reapplication Bag tubing). Press **STOP** and remove Non-Target Cell Bag tubing from valve no. 3. Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no. 3 as described in the installation instructions. Press **RUN** to restart elution sequence within 600 seconds.

8.5 Cell separation performance

8.5.1 Unexpected events: Enrichment programs only

■ The yield of target cells is low

- Target cells were poorly labeled with the reagent:
  - Reagent has expired. Check use-by date. Do not use any reagent after the use-by date.
  - Reagent was not stored properly. Check storage temperature. Do not use any reagent that has been stored improperly (see instructions for use of the reagent).
  - Recommended labeling procedure has not been followed. Refer to sample preparation and separation procedure sections in the user manual.
- Cells were lost during the preparation steps.
  - Cells were removed with the supernatant into Plasma Waste Bag and Wash Waste Bags due to incomplete sedimentation or too early resuspension of the cells, e.g., when the bag was removed from the centrifuge. Compare leukocyte content of the non-labeled cell product and the labeled cell product. Check centrifugation settings for proper centrifugation. Determine cell counts from all waste bags.
  - Buffer did not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5% (w/v), (see section “CliniMACS Materials required” of the respective application).
  - Centrifuge settings were suboptimal. Check centrifugation settings.
  - Centrifuge imbalance or use of brake or asymmetrical loading of centrifuge.

- Analysis was incorrect.
  - Sampling error occured. Check cell suspension for clumped or settled cells. Confirm that representative samples have been taken and repeat analysis.
  - Staining error occured. Check flow cytometry reagents. Repeat staining.
  - Flow cytometer settings were improper. Check instrument settings.

The purity of target cells is low.

- The cell product was stored inappropriately. Preparation and separation of the cell product should be performed immediately after leukapheresis. Keep the cell product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the cell product with autologous plasma. The cell product should not be older than 24 hours when starting the labeling and separation procedure. If the cell product has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).
- The magnetic labeling protocol has not been followed (e.g., incorrect volumes during magnetic labeling). Follow the instructions given for the magnetic labeling (see STEP 1 in the section of the respective application). For troubleshooting purposes determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target cell fraction and contact Miltenyi Biotec Technical Support for advice.
- High numbers of granulocytes contaminated the start product (suboptimal apheresis setting). Dying granulocytes will then bind the CliniMACS Reagent non-specifically which may lead to decreased purity of the target cells.
- Valve malfunction occurred. Eluted target cell fraction has been contaminated by part of the non-target fraction or buffer waste fraction. Inspect tubing placement within the valves to ensure proper functioning. Assess target cell content of the non-target cell fraction and buffer waste. If necessary, pool the target and non-target cell fraction, reduce to suitable volume and repeat the separation with a new tubing set and sufficient new buffer.

Non-specific retention of dead cells from cell product or high non-specific cell losses throughout the procedure

- Buffer does not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5% (w/v), (see section “CliniMACS Materials required” of the respective application).
- The cell product may have been stored inappropriately. Preparation and separation of the cell product should be performed immediately after leukapheresis. Keep the cell product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis with autologous plasma. The cell product should not be older than 24 hours when starting

the labeling and separation procedure. If the cell product has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

- Incomplete sample loading due to clogging of separation column, presystem filter, or pre-column. Check total cell number and depletion efficiency of the remaining cells.

8.5.2 Unexpected events: CD34 SELECTION 1/2 and ENRICHMENT 1.1

The purity of the target cells is low

Elution from the separation column was incomplete.

- Separation program was aborted. Check display screen for error message. Continue with section "Run is aborted before completion of cell separation program" (see section 8.4.3 on page 210).
- Pump failure or valve failure occured. Recover cells from the tubing set following the emergency program described in section 8.4.7. Check volumes of all fractions. Assess target cell content of Buffer Waste Bag and Negative Fraction Bag.
- Tubing to Cell Collection Bag is blocked. Check tubing set for closed clamps, occlusions or kinks.
- Capacity of reagent and/or tubing set was exceeded. Refer to capacity limit of the relevant application.
- Tubing has not been properly inserted. Check all valves for proper tubing insertion.

8.5.3 Unexpected events: CD34 SELECTION 1/2

The purity of the target cells is low

Non-target cells were retained.

- Mobilization of target cells was poor. Very low number of target cells has occurred. Therefore, a low number of contaminating non-target cells (e.g., granulocytes, monocytes, platelets) may lead to decreased purity.
- Insufficient plasma or immunoglobulins were present during magnetic labeling. Follow the instructions given for the magnetic labeling (see STEP 1 of the respective application).

  If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add immunoglobulin to the sample. A final concentration of 1.5 mg/mL is recommended for the efficient blocking of non-specific reagent binding during magnetic labeling.

8.5.4 Unexpected events: ENRICHMENT 1.1

■ The yield of the target cells is low

Incorrect sample parameter input. Cross-check parameter input with analysis results.

8.5.5 Unexpected events: DEPLETION 2.1 and DEPLETION 3.1

■ The depletion efficiency is low

If the depletion efficiency is insufficient, process the remaining sample with a new tubing set and sufficient new buffer. A new labeling of the cells to be depleted should be considered. Determine the cell count and percentage of cells to be depleted and enter actual sample parameters during set-up of the instrument (STEP 2). If visible clumps occur, it may be helpful to filter the cells prior to a new run. Consider that cells may be lost due to clumping and additional filtration.

- Capacity of reagent and/or tubing set was exceeded. Refer to capacity limit of the relevant application.
- Incorrect determination of cells to be labeled by the reagent.
- Incorrect sample parameter input. Cross-check parameter input with analysis results.
- Reduced viability of the cells. Dead cells can bind non-specifically to separation column thereby reducing the labeling capacity of the column, potentially resulting in lower depletion efficiency. Ensure better cell product quality and process product as fresh as possible. Stored products that are older than 24 hours should not be used. If storage is necessary, cell product should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). The cell concentration should not exceed 0.2×10^9/mL. If necessary, dilute the cell product with autologous plasma to achieve optimal cell concentration.

9 Legal notes

9.1 Limited warranty

Except as stated in a specific warranty statement, which may accompany your product, or unless otherwise agreed in writing by a duly authorized representative of Miltenyi Biotec, Miltenyi Biotec's warranty for any product purchased directly from Miltenyi Biotec shall be subject to the terms and conditions of sale under which it was provided to you by the respective Miltenyi Biotec sales organization. The applicable terms and conditions of sale may vary by country and region. These terms and conditions are available on request or at www.miltenyibiotec.com. Nothing herein should be construed as constituting an additional warranty.

For products purchased from third-party retailers or resellers (e.g., purchased from an authorized local Miltenyi Biotec Service Provider), different terms and conditions may apply.

To determine the warranty that came with your product, see your packing slip, invoice, receipt or other sales documentation. Some components of a product combination you purchased may have a shorter warranty than that listed on your packing slip, invoice, receipt or other sales documentation (e.g., goods subject to shelf life and obsolescence).

Miltenyi Biotec's warranty for this product only covers product issues caused by defects in material or workmanship during normal use. It does not cover product issues caused by any other reason, including but not limited to product issues due to use of the product in a manner other than specifically described in this manual, for example: inappropriate or improper use; incorrect assembly or installation by an operator or a third party; reasonable wear and tear; negligent or incorrect operation, handling, storage, servicing, or maintenance; non-adherence to the operating instructions; unauthorized modification of or to any part of this product; or use of inappropriate consumables, accessories, or work materials.

Miltenyi Biotec's warranty does not cover products sold AS IS or WITH ALL FAULTS or consumables. Nothing herein should be construed as constituting an additional warranty.

Miltenyi Biotec must be informed immediately, if a claim is made under such warranty. If a material or manufacturing defect occurs within the warranty period, Miltenyi Biotec will take the appropriate steps to restore the full usability of the instrument.

Limitation on damages Miltenyi Biotec shall not be liable for any incidental or consequential damages for breach of any express or implied warranty or condition on this product.

Some countries/states or jurisdictions do not allow the exclusion or limitation of incidental or consequential damages, so the above limitations or exclusions may not apply to you. This warranty statement gives you specific legal rights and you may have other rights, which vary from state to state or jurisdiction to jurisdiction.

9.2 Trademarks

CliniMACS, MACS, MACSmix, the Miltenyi Biotec logo, and MiniMACS are registered trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only.

Miltenyi Biotec

Germany/Austria
Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
+49 2204 8306-0
+49 2204 85197
macsde@miltenyi.com

USA/Canada
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602
USA
800 FOR MACS
+1 866 811 4466
+1 877 591 1060
macsus@miltenyi.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 11, 2 Eden Park Drive
Macquarie Park NSW 2113
Australia
+61 2 8877 7400
+61 2 9889 5044
macsau@miltenyi.com

Benelux
Miltenyi Biotec B.V.
Dellaertweg 9C
2316 WZ Leiden
The Netherlands
macsnl@miltenyi.com
Customer service The Netherlands
0800 4020120
0800 4020100
Customer service Belgium
0800 94016
0800 99626
Customer service Luxembourg
800 24971
800 24984

China
Miltenyi Biotec Technology &
Trading (Shanghai) Co., Ltd.
Room A401, 4/F
No. 1077, Zhangheng Road
Pudong New Area
201203 Shanghai
P.R. China
+86 21 6235 1005-0
+86 21 6235 0953
macscn@miltenyi.com.cn

France
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris
France
+33 1 56 98 16 16
macsfr@miltenyi.com

Hong Kong
Miltenyi Biotec Hong Kong Ltd.
Unit 301, Lakeside 1
No. 8 Science Park West Avenue
Hong Kong Science Park
Pak Shek Kok, New Territories
Hong Kong
+852 3751 6698
+852 3619 5772
macshk@miltenyi.com.hk

Italy
Miltenyi Biotec S.r.l.
Via Paolo Nanni Costa, 30
40133 Bologna
Italy
+39 051 6 460 411
+39 051 6 460 499
macsit@miltenyi.com

Japan
Miltenyi Biotec K.K.
NEX-Eitai Building 5F
16-10 Fuyuki, Koto-ku
Tokyo 135-0041
Japan
+81 3 5646 8910
+81 3 5646 8911
macsjp@miltenyi.com

Nordics and Baltics
Miltenyi Biotec Norden AB
Medicon Village
Scheeletorget 1
223 81 Lund
Sweden
macsse@miltenyi.com
Customer service Sweden
0200 111 800
+46 280 72 99
Customer service Denmark
80 20 30 10
+46 46 280 72 99
Customer service
Norway, Finland, Iceland,
and Baltic countries
+46 46 280 72 80
+46 46 280 72 99

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd.
438B Alexandra Road, Block B
Alexandra Technopark
#06-01
Singapore 119968
+65 6238 8183
+65 6238 0302
macssg@miltenyi.com

South Korea
Miltenyi Biotec Korea Co., Ltd.
Donggeuk 7F
562 Nonhyeon-ro
Gangnam-gu
Seoul 06136
South Korea
+82 2 555 1988
+82 2 555 8890
macskr@miltenyi.com

Spain
Miltenyi Biotec S.L.
C/Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón (Madrid)
Spain
+34 91 512 12 90
+34 91 512 12 91
macses@miltenyi.com

Switzerland
Miltenyi Biotec Swiss AG
Gibelinstrasse 27
4500 Solothurn
Switzerland
+41 32 623 08 47
+49 2204 85197
macsch@miltenyi.com

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR
United Kingdom
+44 1483 799 800
+44 1483 799 811
macsuk@miltenyi.com

www.miltenyibiotec.com

210-003-187/03 | Issued: 2023-02

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

CliniMACS, MACS, MACSmix, the Miltenyi Biotec logo, and MiniMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. Copyright © 2023 Miltenyi Biotec and/or its affiliates. All rights reserved.