

Miltenyi Biotec

Memory T cells – Depletion of CD45RA+ cells

CliniMACS® Plus CD45RA and CliniMACS Prodigy® PB-45RA Systems

Miltenyi Biotec offers clinical-grade CD45RA+ cell depletion from leukapheresis material for the manufacture of memory T cell products. The process follows the same depletion principle on either the CliniMACS Plus or the CliniMACS Prodigy Instrument.

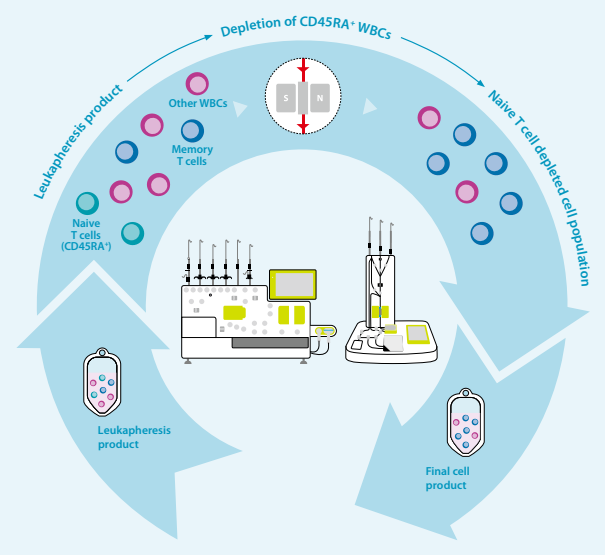
The benefit of memory T cell products

Donor-derived CD45RA-depleted cell products are used in allogeneic hematopoietic stem cell transplantation (HSCT) to leverage the immunity that memory T cells provide against viral infections that the donor has experienced during a lifetime. Prior to their clinical application, studies in mouse models showed that the potential for alloreactivity is high in the naive T cell compartment and significantly reduced in the memory T cell compartment.[1]

CD45RA-depleted cell products, manufactured with the CliniMACS CD45RA System, are used to treat opportunistic infections and enhance immune reconstitution in allogeneic HSCT.[2,3,4] CD45RA is an isoform of the CD45 family that is present on naive T cells but not on CD45RO+ central and effector memory T cells.[5,6] Depleting CD45RA+ cells also removes approximately 100% of B cells, most NK cells, and other white blood cell types, such as CD34+ stem cells, from mobilized leukapheresis products.[7,8]

CD45RA is not a typical T cell marker but is suited to deplete potentially alloreactive cells from a product in one step, leaving very specific beneficial memory T cells. Thus, CD45RA depletion is a key production option for clinical research applications.[9,10]

Principle of CliniMACS CD45RA Depletion



Application on the CliniMACS Plus CD45RA System

The CliniMACS Plus CD45RA System is a semi-automated procedure, with magnetic labeling and cell preparation performed in blood bags and bag centrifuges. The prepared bags are then connected to a closed and sterile system, where the magnetically labeled CD45RA-positive cells are automatically removed from suspension as they pass through a magnetic column. The unlabeled cells (target cells) flow through the column and, after several automated washing steps, are collected in the Cell Collection Bag. The instrument disposes most of the liquid into the Buffer Waste Bag and, upon disengaging the magnet, releases the captured cells from the separation column.

Specifications

Technology:	CliniMACS Plus Instrument
Program name:	Depletion 3.1
Starting material:	Leukapheresis products
Process capacity Total:	50×10^9 white blood cells (WBC)
CD45RA$^+$ depletion	20×10^9 CD45RA$^+$ cells
Sample volume:	Up to 200 mL
Process time:	4–5 hours
Hands-on time:	2 hours

Materials and equipment

CliniMACS Products	Amount required
CliniMACS Plus Instrument	1
CliniMACS CD45RA Reagent	1 vial
CliniMACS Depletion Tubing Set	1 set
CliniMACS PBS/EDTA Buffer	3×1 L

Additional materials	Amount required
Human serum albumin (HSA)	to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
600 mL transfer bags	3–4
Pre-system filter	1
Appropriate syringes (1 mL, 10 mL, 20 mL, 50 mL) and hypodermic gauge needles, locking forceps, sample tubes	

Required equipment
Uninterruptible power supply unit
Laminar flow hood
Orbital rotator
Bag centrifuge
Plasma extractor
Tabletop balance
Tubing heat sealer
Sterile docking device
Cell counter

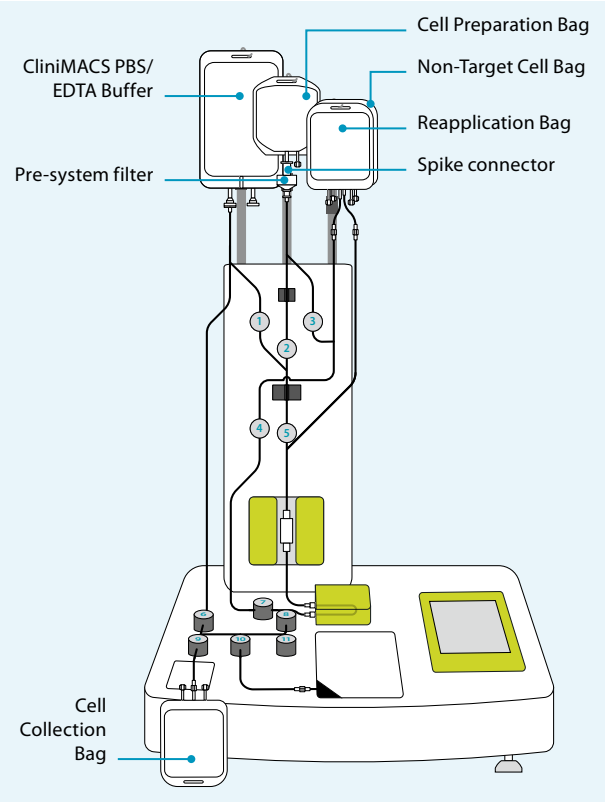


Figure 1: CliniMACS Plus Instrument with CliniMACS Depletion Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag.

Performance data

Depletion efficacy

Cell type	Mean	Range
Naive T cells[9]	4.7	
CD45RA$^+$ cells[10]	4.4	3.4–4.7
CD45RA$^+$ cells[3]	3.6	2.3–3.95

Log depletion of CD45RA$^+$ T cells and naive T cells using the CliniMACS Plus Platform. Published data.

Application on the CliniMACS Prodigy PB-45RA System

The procedure to deplete CD45RA-expressing cells on the CliniMACS Prodigy PB-45RA System is fully automated and requires only minimal preparation. Once the process is initiated, all steps are fully enclosed in the system and automated, including washing, magnetic labeling, and magnetic separation of $CD45RA^+$ cells. The CD45RA-negative target cells are collected into a separate bag suspended in target cell solution (e.g., physiological sodium chloride solution), and are ready for further applications.

The procedure includes an automated red blood cell (RBC) removal sequence based on an integrated camera system. Thus, whole blood samples can be processed directly.

Specifications

Technology:	CliniMACS Prodigy
Program name:	PB-45RA Depletion
Starting material:	Leukapheresis products Whole blood
Process capacity Total:	5×10^9 white blood cells (WBC)
$CD45RA^+$ depletion	1.8×10^9 $CD45RA^+$ cells
Sample volume:	50–280 mL
Process time:	3–4 hours
Hands-on time:	<1 hour

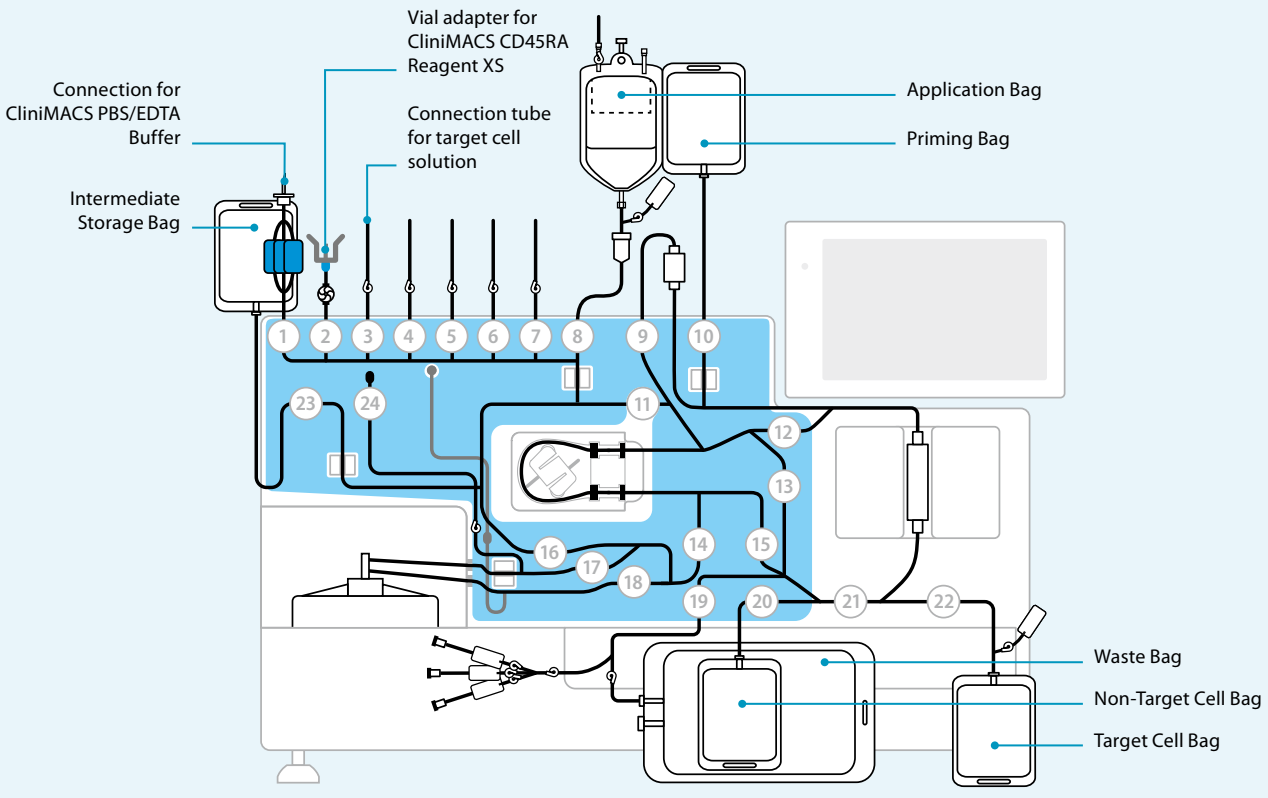
Products

CliniMACS Products	Amount required
CliniMACS Prodigy Instrument	1
CliniMACS CD45RA Reagent XS	1 vial
CliniMACS Prodigy TS 510	1 set
CliniMACS PBS/EDTA Buffer	1×3 L

Additional materials	Amount required
Human serum albumin (HSA)	to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
Physiological saline solution (NaCl 0.9%), suitable for infusion (or equivalent)	Bag with 2 L
Appropriate syringes (10 mL, 50 mL) and hypodermic 20-gauge needles, sample tubes	

Required equipment
Uninterruptible power supply unit
Sterile docking device
Cell counter

CliniMACS Prodigy TS 510 setup for the PB-45RA System



Performance data

Depletion efficacy

Starting material	Mean	Range
Whole blood (N = 3) Leukapheresis product (N = 3)	5.0	3.9–5.7

Log depletion of CD45RA$^+$ T cells obtained with CliniMACS Prodigy technology.[11]

References

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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

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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 devices for their respective intended use, unless otherwise stated. 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 System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS 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 System.
In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (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.
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