Miltenyi Biotec

CliniMACS Prodigy® LP-25 Pre-Enrichment

Enrichment and fluorescent labeling of human (naive) regulatory T cells

Application

This process overview sheet provides the workflow for the magnetic pre-enrichment and fluorescent labeling of $CD25^+$ cells from leukapheresis material for subsequent flow cytometry sorting of (naive) regulatory T (Treg) cells, e.g., by using the MACSQuant® Tyto®.
The workflow is designed to be automated on the CliniMACS Prodigy.
Further, it gives an overview of materials needed, the workflow process, and illustrates the configuration of the required CliniMACS Prodigy Tubing Set.

Specifications

Program name:	LP-25 Pre-Enrichment
Starting material:	Leukapheresis
Total cells:	≤ 1×10^{10} white blood cells (WBC)
Sample volume:	50–280 mL
Elution volume:	10 or 20 mL (subsequent automated dilution possible)
Process time:	4–5 hours

Materials required

CliniMACS® Products	Amount required
CliniMACS Prodigy	1 unit
CliniMACS Prodigy TS 510	1 piece
CliniMACS PBS/EDTA Buffer (2×3 L)	3 L
CliniMACS Anti-Biotin Reagent	1 vial

Additional materials	Amount required
Single Vial Adapter	3*(2) pieces
Double Vial Adapter	1 piece
Sterile Filter Adapter	1 piece
MACS GMP CD25 PE-Biotin	1 vial
MACS GMP CD4-VioBlue	1 vial
MACS GMP CD127-APC	1 vial
MACS GMP CD45RA-FITC*	1 vial
MACS GMP PBS/$MgCl_2$ Buffer (3×1 L)	1 L
MACS GMP CD127-FITC	1 vial
MACS GMP CD45RA-APC*	1 vial
MACS GMP Tytonase	1 vial

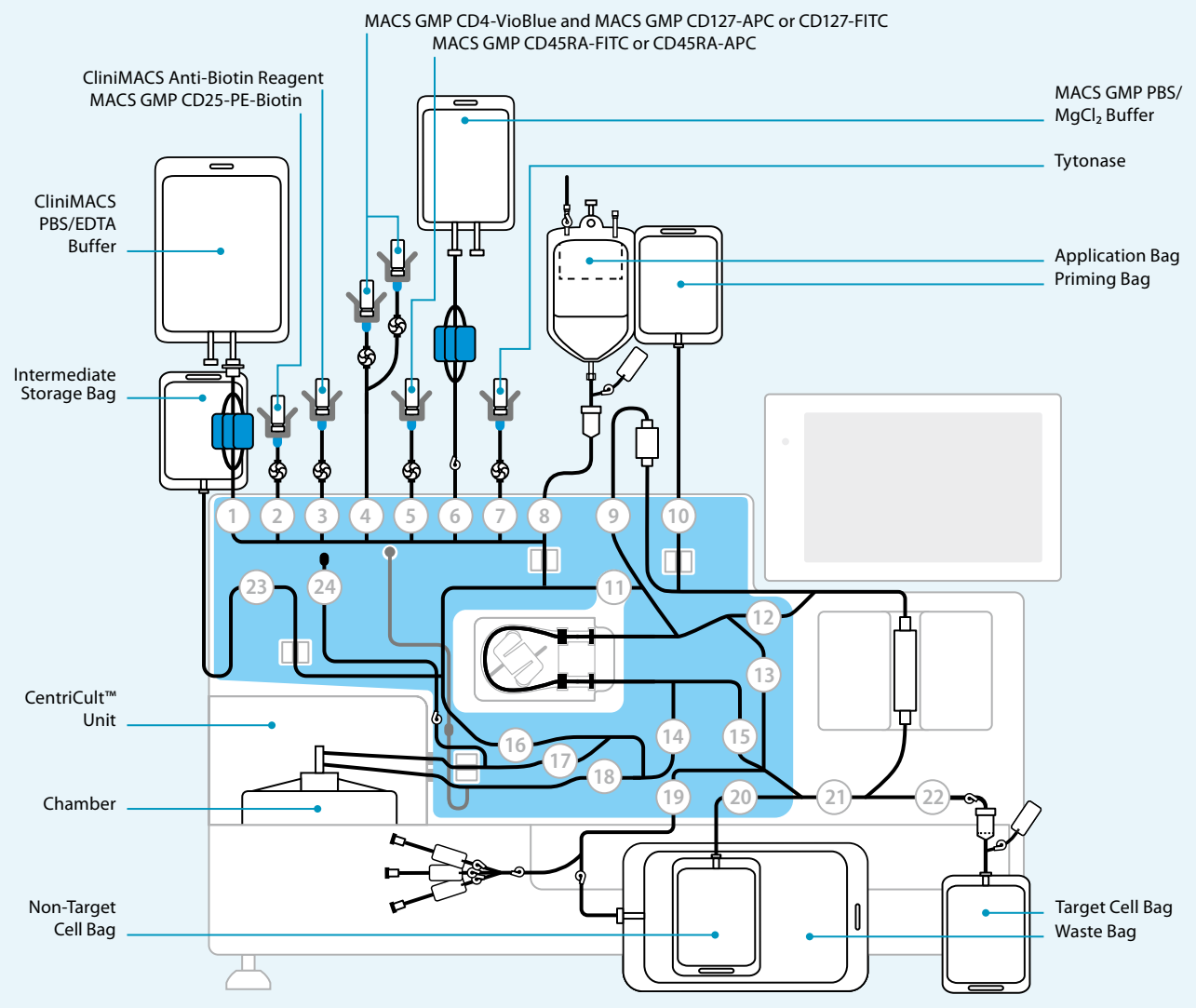
*Only required if MACS® GMP CD45RA-FITC/-APC is included in the LP-25 Pre-Enrichment process.
For availability in your country please contact your local representative.
In the US, this application is for research use only.
For complete regulatory and legal notices please refer to the last page.

Workflow of the CliniMACS Prodigy LP-25 Pre-Enrichment Process

Pre-process	Process parameter input ▼ Tubing set modification, installation and priming ▼
Enrichment	Sample loading ▼ Cell washing ▼ Cell labeling with MACS GMP CD25-PE-Biotin ▼ Cell washing (optional) ▼ Cell labeling with CliniMACS Anti-Biotin Reagent CR/GMP ▼ Cell washing ▼ Column loading ▼
Labeling	Mixing of MACS GMP Fluorescent Antibodies (e.g. CD4-VioBlue®, CD127-APC, and CD45RA-FITC (optional)) in the Priming Bag ▼ Column loading ▼
Elution	Preparation of elution buffer in the Priming Bag and storage in the Intermediate Storage Bag ▼ Elution of pre-enriched and fluorescently labeled $CD25^+$ cells ▼
Post-process	Tubing set de-installation

Approx. 4–5 hours

CliniMACS Prodigy TS 510 setup



Performance data

White blood cells obtained via leukapharesis were pre-enriched in the CliniMACS Prodigy. A MACS Peripheral Blood ¼ Leukopak (Miltenyi Biotec, # 150-000-451*) containing approximately 2.5×10^9 cells was loaded onto the CliniMACS Prodigy and parameters were set using CliniMACS LP-25 Pre-enrichment Process. CliniMACS Prodigy-enriched sample was first analyzed for expression of CD4 (y-axis) and CD25 (x-axis), and then plotted to show CD25-PE (y-axis) vs CD127-APC (x-axis) expression, using the MACSQuant Analyzer 10. The results are depicted (fig. 1). After pre-enrichment on the CliniMACS Prodigy, Tregs ($CD4^+CD25^+CD127^{dim/-}$) were enriched to a purity of 38%.

*Product only available in USA/Canada

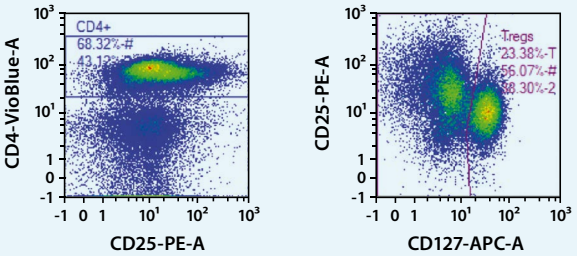


Figure 1: Purity of Tregs ($CD4^+CD25^+CD127^{dim/-}$) after pre-enrichment on the CliniMACS Prodigy. Target cells were enriched to a purity of 38%.

Miltenyi Biotec

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

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

130-117-649.04