



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<sup>+</sup> 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 ClinMACS Prodigy.

Further, it gives an overview of materials needed, the workflow process, and illustrates the configuration of the required ClinMACS Prodigy Tubing Set.

### Specifications

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

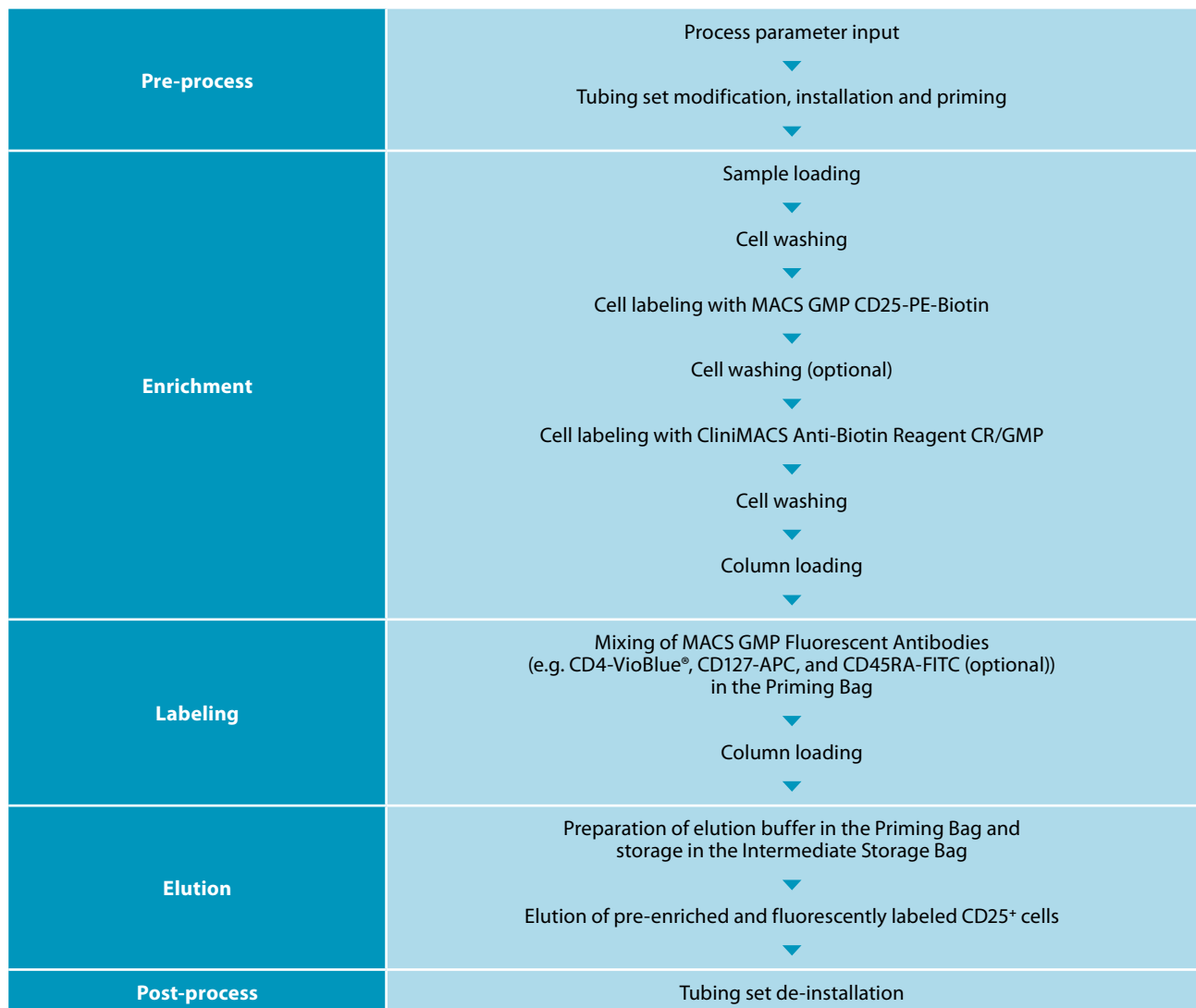
### Materials required

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 <sub>2</sub> Buffer (3×1 L)	1 L
MACS GMP CD127-FITC	1 vial
MACS GMP CD45RA-APC*	1 vial
MACS GMP Tytonase	1 vial

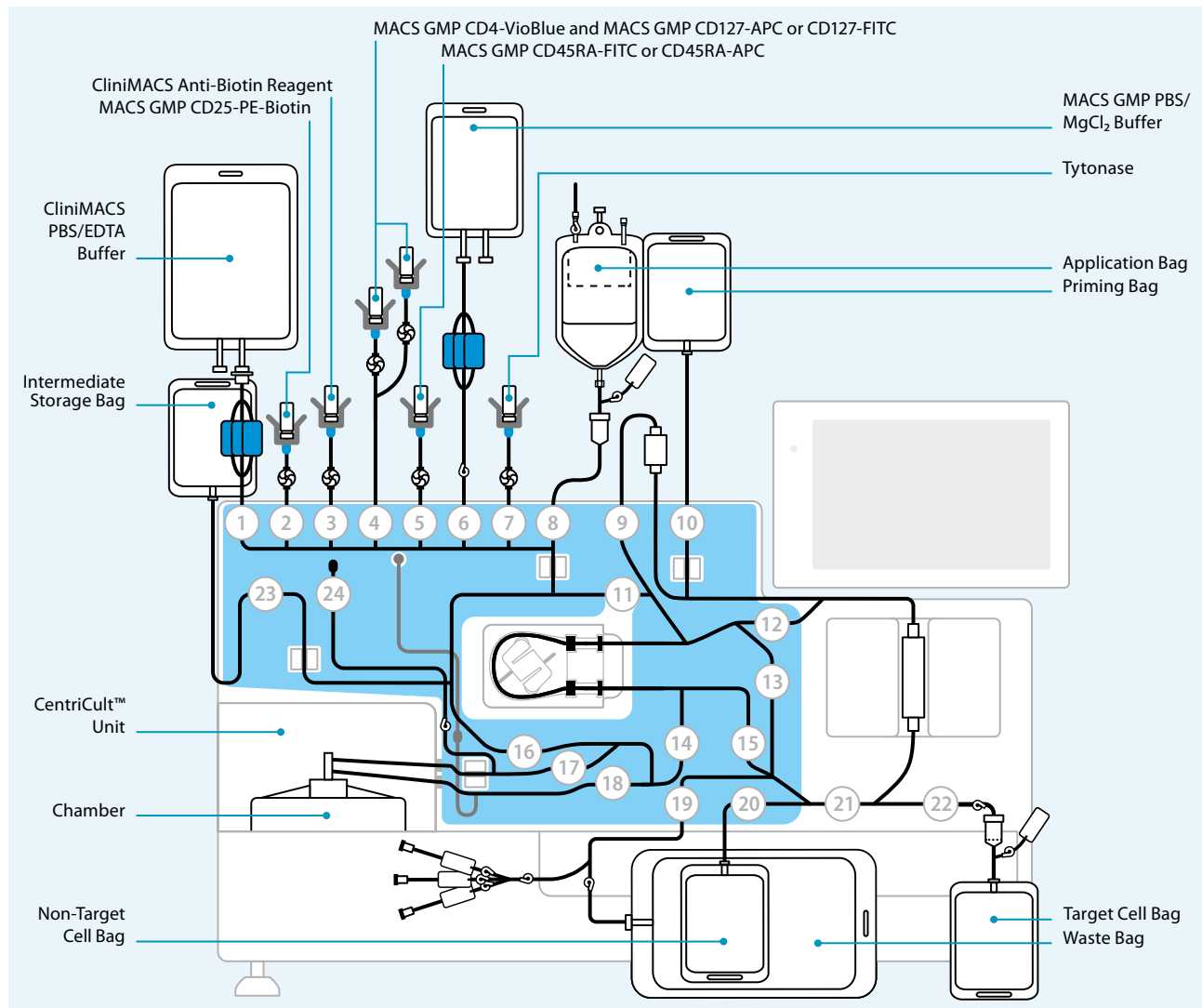
\*Only required if MACS® GMP CD45RA-FITC/-APC is included in the LP-25 Pre-Enrichment process.  
For availability in your country please contact your local representative.  
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## Workflow of the CliniMACS Prodigy LP-25 Pre-Enrichment Process



**Approx. 4–5 hours**

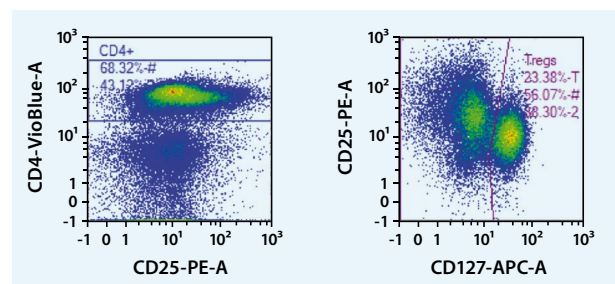
## CliniMACS Prodigy TS 510 setup



## Performance data

White blood cells obtained via leukapheresis were pre-enriched in the CliniMACS Prodigy. A MACS Peripheral Blood ¼ Leukopak (Miltenyi Biotec, # 150-000-451\*) containing approximately  $2.5 \times 10^9$  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<sup>+</sup>CD25<sup>+</sup>CD127<sup>dim/-</sup>) were enriched to a purity of 38%.

\*Product only available in USA/Canada



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



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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. CliniMACS GMP MicroBeads are for *ex vivo* cell processing only and are not intended for human *in vivo* applications.

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