

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

LP-25 Pre-Enrichment

Application

This Application 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 fully automated on the CliniMACS Prodigy[®].

Further, the application sheet gives an outline of materials needed, the workflow process, a review of the set-up of the tubing set, and the expected performance data.

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 liters
CliniMACS Anti-Biotin Reagent	1 vial

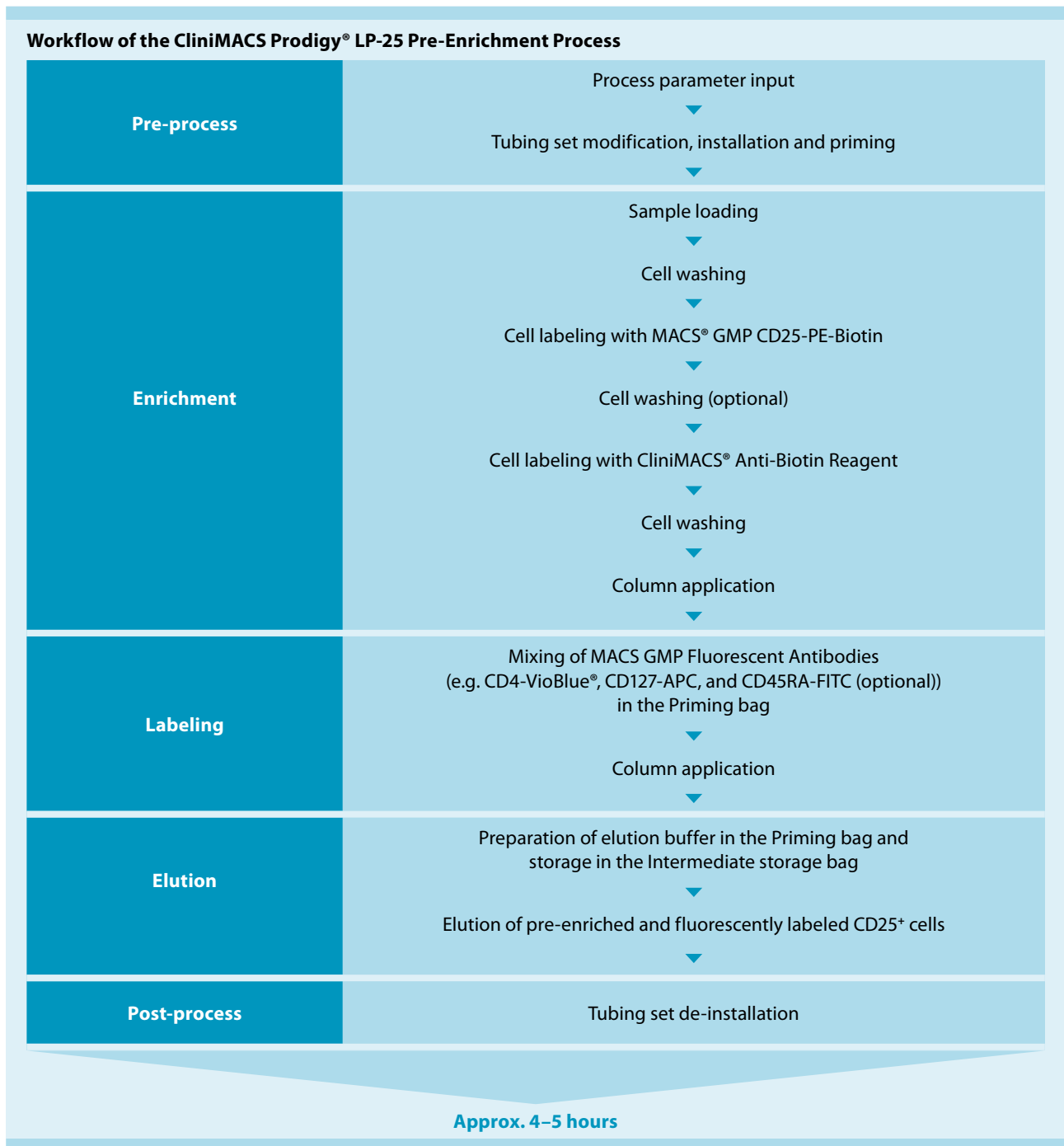
Additional products	Amount required
Single Vial Adapter	3*(2) pieces
Double Vial Adapter	1 piece
Sterile Filter Adapter	1 piece
MACS GMP CD25 PE-Biotin	1 vial
MACS GMP CD4-VioBlue [®]	1 vial
MACS GMP CD127-APC	1 vial
MACS GMP CD45RA-FITC*	1 vial
MACS GMP PBS/MgCl ₂ Buffer (3×1 L)	1 liter
MACS GMP CD127-FITC	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.

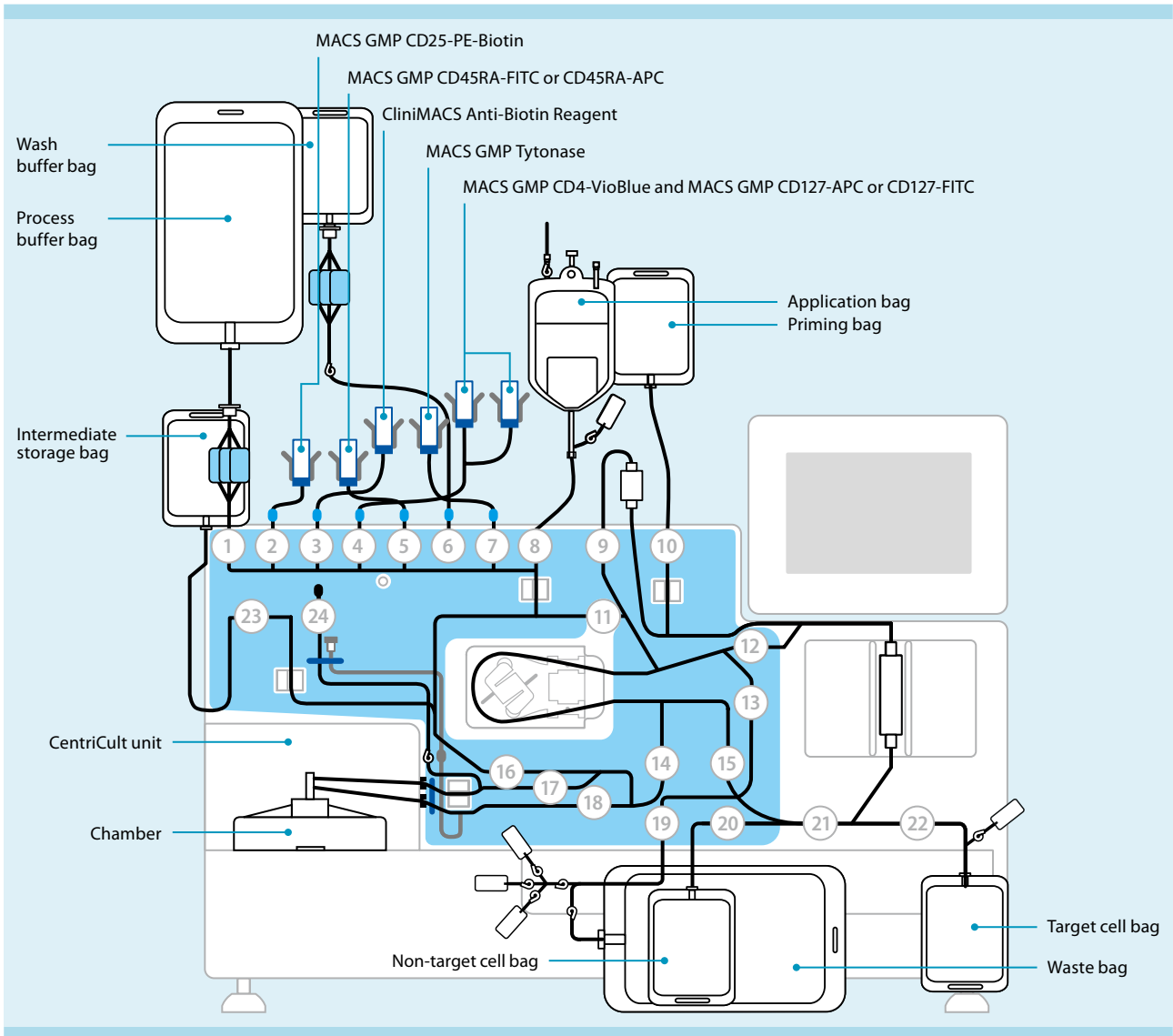
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Process overview



CliniMACS Prodigy® TS 510 setup



Performance data

Data from four independent experiments are shown below.

Treg cells (CD4⁺CD25⁺CD127^{dim/-})

N=4	Starting material			After pre-enrichment			
	Total WBC	Treg cells		Total WBC	Treg cells		
(%)		Total	(%)		Total	Recovery (%)	
Mean	6.58×10 ⁹	1.7	1.08×10 ⁸	2.42×10 ⁸	24.2	5.70×10 ⁷	52.7
SD	1.18×10 ⁹	0.2	7.78×10 ⁶	4.59×10 ⁷	4.8	9.34×10 ⁶	6.5

Naive Treg cells (CD4⁺CD25⁺CD127^{dim/-}CD45RA⁺)

N=4	Starting material			After pre-enrichment			
	Total WBC	Naive Treg cells		Total WBC	Naive Treg cells		
(%)		Total	(%)		Total	Recovery (%)	
Mean	6.58×10 ⁹	0.4	2.6×10 ⁷	2.42×10 ⁸	5.9	1.31×10 ⁷	50.1
SD	1.18×10 ⁹	0.1	9.3×10 ⁶	4.59×10 ⁷	3.5	4.98×10 ⁶	4.5



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