



Miltenyi Biotec



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

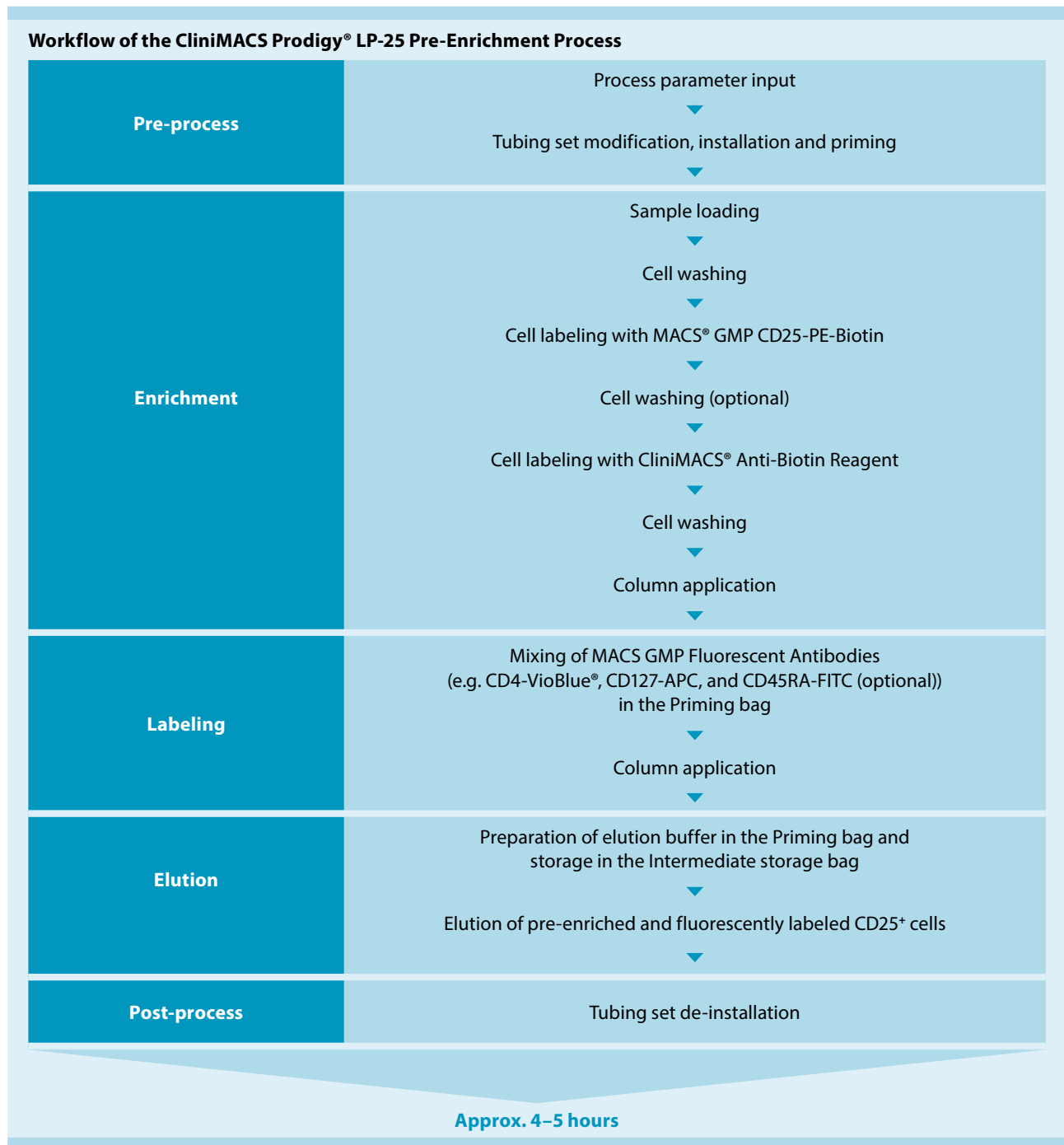
* Only required if MACS[®] GMP CD45RA-FITC/-APC is included in the LP-25 Pre-Enrichment process.

For availability in your country please contact your local representative.

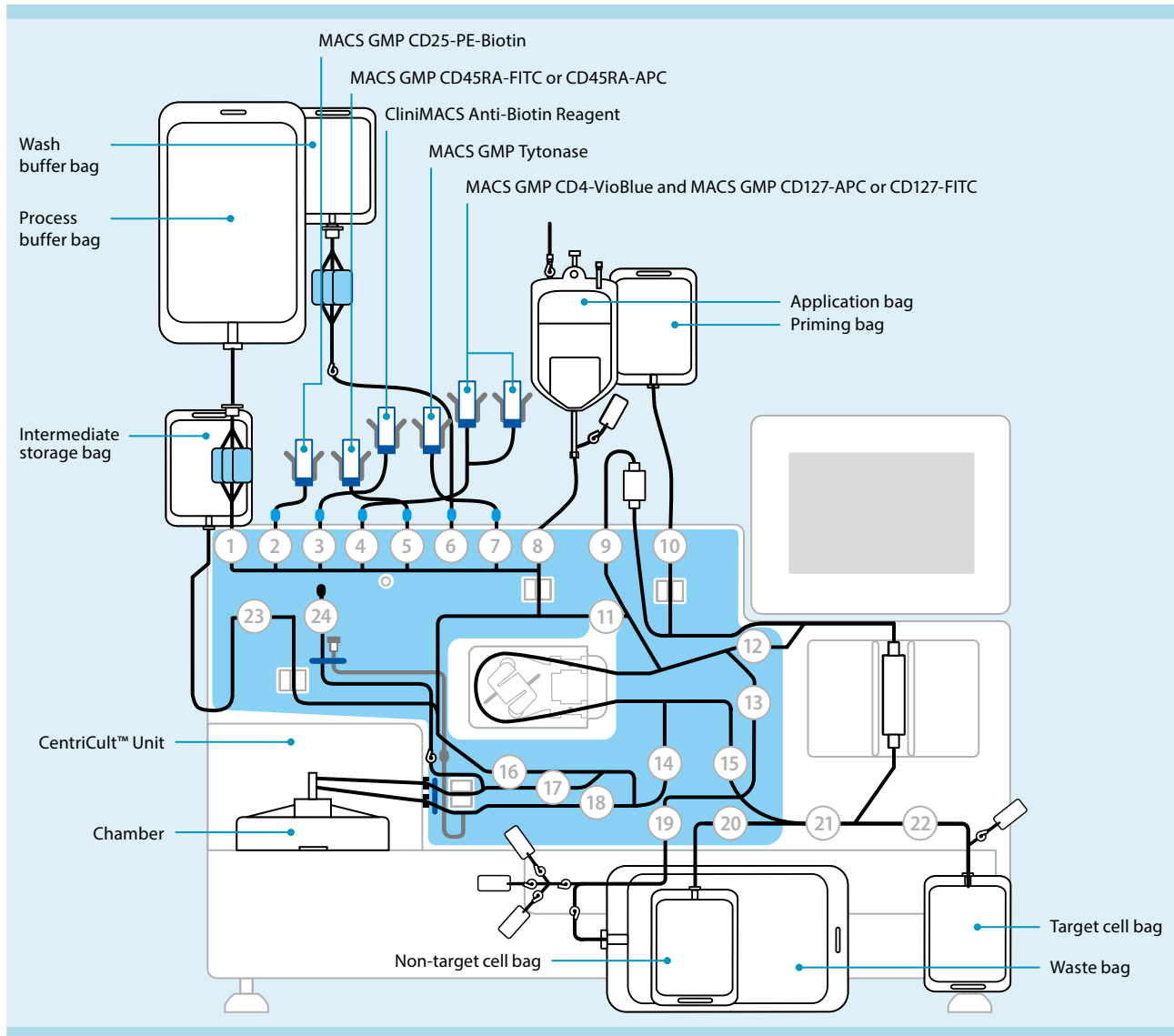
In the US, this application is for research use only.

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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



Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG

Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macsde@miltenyi.com

Miltenyi Biotec Inc.

2303 Lindbergh Street
Auburn, CA 95602-9562, USA
Phone 800 FOR MACS
Phone +1 530 888 8871
Fax +1 877 591 1060
macsus@miltenyi.com

Miltenyi Biotec Australia Pty. Ltd.

Phone +61 2 8877 7400
macsau@miltenyi.com

Miltenyi Biotec B.V. (Benelux)

macsnl@miltenyi.com
**Customer service
The Netherlands**
Phone 0800 4020120
Customer service Belgium
Phone 0800 94016
Customer service Luxembourg
Phone 800 24971

Miltenyi Biotec Technology & Trading (Shanghai) Co., Ltd. (P.R. China)

Phone +86 21 6235 1005
macscn@miltenyi.com

Miltenyi Biotec SAS (France)

Phone +33 1 56 98 16 16
macsfr@miltenyi.com

Miltenyi Biotec S.r.l. (Italy)

Phone +39 051 6 460 411
macsit@miltenyi.com

Miltenyi Biotec K.K. (Japan)

Phone +81 3 5646 8910
macsjp@miltenyi.com

Miltenyi Biotec Norden AB (Nordics and Baltics)

macsse@miltenyi.com
Customer service Sweden
Phone 0200 111 800

Customer service Denmark

Phone 80 20 30 10

Customer service Norway, Finland, Iceland, and Baltic countries

Phone +46 46 280 72 80

Miltenyi Biotec Asia Pacific Pte Ltd (Singapore)

Phone +65 6238 8183
macssg@miltenyi.com

Miltenyi Biotec Korea Co., Ltd. (South Korea)

Phone +82 2 555 1988
macskr@miltenyi.com

Miltenyi Biotec S.L. (Spain)

Phone +34 91 512 12 90
macses@miltenyi.com

Miltenyi Biotec Swiss AG (Switzerland)

Phone +41 32 623 08 47
macsch@miltenyi.com

Miltenyi Biotec Ltd. (UK)

Phone +44 1483 799 800
macsuk@miltenyi.com

www.miltenyibiotec.com

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