

# Depletion of CD3<sup>+</sup> cells with and without enrichment of CD56<sup>+</sup> cells

CliniMACS Prodigy® LP-3-56 System

## Application

This application sheet gives an overview of the automated workflow for the *in vitro* depletion of human CD3 expressing cells with optional subsequent *in vitro* enrichment of human CD56 expressing cells from unmobilized leukapheresis products.

This workflow is designed to be fully automated on the CliniMACS Prodigy. Furthermore, the application sheet gives an outline of the required materials, the workflow process, the configuration of the CliniMACS Prodigy Tubing Set and expected performance data from internal runs.

# **Specifications**

Program name:	LP-3-56 Separation
Starting material:	Unmobilized leukapheresis product
Process capacity:	
Total:	$\leq$ 40×10 <sup>9</sup> white blood cells (WBC)
CD3 <sup>+</sup> cell depletion:	9.6×10 <sup>9</sup> CD3⁺ cells
CD56 <sup>+</sup> cell enrichment:	4.5×10 <sup>9</sup> CD56 <sup>+</sup> cells
Sample volume:	50–600 mL
Process time:	
CD3 <sup>+</sup> cell depletion:	3.5–5 hours
CD3 <sup>+</sup> cell depletion +	
CD56 <sup>+</sup> cell enrichment:	7.5–9.5 hours

## Products

CliniMACS® Products	Amount required			
	Use case 1	Use case 2	Use case 3	
CliniMACS Prodigy	1	1	1	
CliniMACS CD3 Reagent	1 vial	1 vial	1 vial	
CliniMACS CD56 Reagent	1 vial			
CliniMACS Prodigy TS 320	1 set	1 set	1 set	
CliniMACS PBS/EDTA Buffer	9 liters	6 liters	6 liters	

Additional materials	Amount required				
	Use case 1	Use case 2	Use case 3		
Human serum albumin (HSA)	to be added to the CliniMACS PBS/ EDTA Buffer and to the formulation solution to a final concentration of 0.5% (w/v)				
5% IgG solution	10 mL	10 mL	10 mL		
Formulation Solution (e.g. CliniMACS Formulation Solution or NaCl + 0.5% HSA)	1,000 mL	-	2,000 mL		
Rinsing solution (aqua bidest. for injection use)	1,000 mL	-	-		
Appropriate syringes (10 mL and 50 mL)	10–50 mL	10–50 mL	10–50 mL		
Plasma transfer set	4	1	2		

#### **Optional materials**

Uninterruptible power supply unit

Sterile docking device

Cell counter

### Process overview: Case selection

Depending on your desired cell product, three options (use cases) for cell separation are available:

#### Use case 1: CD3<sup>+</sup> cell depletion and CD56<sup>+</sup> cell enrichment

Select this option to run the complete process, including CD3<sup>+</sup> cell depletion, rinsing of tubing set with rinsing solution and subsequent CD56<sup>+</sup> cell enrichment. The target cells (CD3<sup>-</sup> and CD56<sup>+</sup> cells) are eluted in formulation solution, ready for downstream applications.

#### Use case 2: CD3<sup>+</sup> cell depletion (process buffer)

Choose this option if you only want to perform a CD3<sup>+</sup> cell depletion from leukapheresis product. Target cells (CD3<sup>-</sup> cells) are separated and collected in process buffer, ready for further applications.

#### Use case 3: CD3<sup>+</sup> cell depletion (formulation solution)

Like in case 2, only CD3<sup>+</sup> cell depletion from leukapheresis product is performed in this option. However, target cells  $(CD3^-)$  are separated and collected in formulation solution, ready to be used in further applications.

	Case 1	Case 2	Case 3	
Pre-process	Tubing set installation	Tubing set installation	Tubing set installation	
	Tubing set priming	Tubing set priming	Tubing set priming	
	Depletion preparation with process buffer	Depletion preparation with process buffer	Depletion preparation with formulation solution	
Depletion	CD3 <sup>+</sup> cell depletion with process buffer	CD3 <sup>+</sup> cell depletion with process buffer	CD3 <sup>+</sup> cell depletion with formulation solution	
Rinsing	Chamber and column rinsing	-	-	
Enrichment	Enrichment preparation with process buffer	-	-	
	CD56 <sup>+</sup> cell enrichment in formulation solution	-	-	
Cell product	product CD56+CD3 <sup>-</sup> cells in formulation solution		CD3 <sup>-</sup> cells in formulation solution	
Post-process	Tubing set de-installation	Tubing set de-installation	Tubing set de-installation	
Process time (approx.)	7.5–9.5 hours	3.5–5 hours	3.5–5 hours	

# Principle of the CliniMACS Prodigy LP-3-56 System workflow



# CliniMACS Prodigy TS 320 setup for the LP-3-56 System



# Performance data

N=9		Starting material					
	WBC		Target cells (total CD3⁻CD56⁺)				
	Total	Viability (%)	(%)	Total			
Mean	9.57×10 <sup>9</sup>	94.3	8.8	9.83×10 <sup>8</sup>			
SD	4.67×10 <sup>9</sup>	3.8	3.7	8.62×10 <sup>8</sup>			

N=9	Final cell product								
	WBC		Target cells (total CD3⁻CD56⁺)		Monocytes (CD14 <sup>+</sup> )		T cells (CD3⁺CD14⁻)	B cells (CD19⁺CD14⁻)	
	Total	Viability (%)	(%)	Total	Recovery (%)	%	Total	-logP depletion	-logP depletion
Mean	3.80×10 <sup>8</sup>	93.1	93.8	3.65×10 <sup>8</sup>	41.3	5.2	1.37×10 <sup>7</sup>	4.2	2.6
SD	3.47×10 <sup>8</sup>	6.0	6.3	3.46×10 <sup>8</sup>	8.1	4.3	9.54×10 <sup>6</sup>	0.1	0.2

The table shows the results from nine independent unmobilized apheresis products performing the combined CD3<sup>+</sup> cell depletion and CD56<sup>+</sup> cell enrichment process (use case 1). The mean -logP depletion is 4.2 and 2.6 for T cells and B cells, respectively. High purities of CD3<sup>-</sup>CD56<sup>+</sup> target cells were achieved with an average recovery of 41.3%. The mean viability of the final cell product was 93.1% (SD: Standard deviation). Data derived from our internal experiments.



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.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS GMP Products are for research use and *ex vivo* cell culture processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative. MACS GMP Products are manufactured and tested under a quality system certified to ISO 13485 and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. 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. CliniMACS GMP MicroBeads are for research use and ex vivo cell processing only.

CliniMACS GMP MicroBeads are for research use and *ex vivo* cell processing only. CliniMACS MicroBeads are for research use only and not for human therapeutic or diagnostic use. CliniMACS, CliniMACS Prodigy, and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.