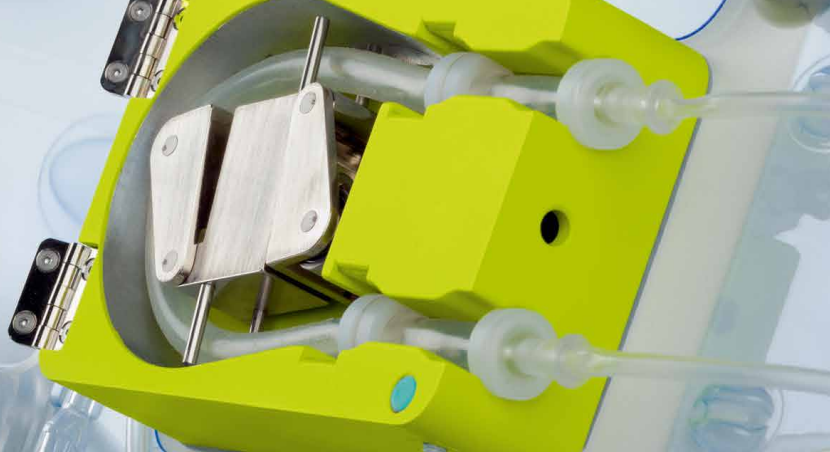




Miltenyi Biotec



CliniMACS Prodigy® LP-3-56 System

Depletion of CD3⁺ and/or enrichment of CD56⁺ cells

Application

This application sheet provides the workflows for the magnetic depletion of CD3⁺ cells, the magnetic enrichment of CD56⁺ cells from leukapheresis and the magnetic enrichment of CD56⁺ cells from CD3⁺ pre-depleted leukapheresis.

The 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, a review of the set-up of the tubing set and the expected performance data.

Specifications

Program name:	LP-3-56 Separation
Starting material:	Leukapheresis or CD3 ⁺ pre-depleted leukapheresis
Total cells:	≤40×10 ⁹ white blood cells (WBC)
Process capacity for CD3⁺ cell depletion:	9.6×10 ⁹ CD3 ⁺ cells
Process capacity for CD56⁺ cell enrichment:	4.5×10 ⁹ CD56 ⁺ cells
Sample volume:	50–660 mL
Elution volume:	120–425 mL

Products

CliniMACS® Products	Amount required
CliniMACS Prodigy	1 unit
CliniMACS CD3 Reagent ¹	1 vial
CliniMACS CD56 Reagent ²	1 vial
CliniMACS Prodigy TS 310	1 set
CliniMACS PBS/EDTA Buffer (3×3 L)	9 L

Additional materials	Amount required
Luer/Spike Interconnector ¹	1 piece
Transfer Set Coupler/Coupler	2–4 sets
Transfer Bag 150 mL ¹	1 bag
5% IgG solution ¹	10 mL

Material not provided by Miltenyi Biotec

Additional materials	Amount required
1,000 mL Infusion solution (e.g. NaCl + 0.5% HSA)	1 bag
1,000 mL Rinsing solution (aqua bidest. for injection use) ¹	1 bag
5,000 mL Transfer bag as waste bag	1 bag
Appropriate syringes (10 mL and 50 mL)	
Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)	

Optional material

- Uninterruptible power supply unit
- Sterile docking device
- Cell counter

¹Required if CD3⁺ cell depletion is to be performed

²Required if CD56⁺ cell enrichment is to be performed

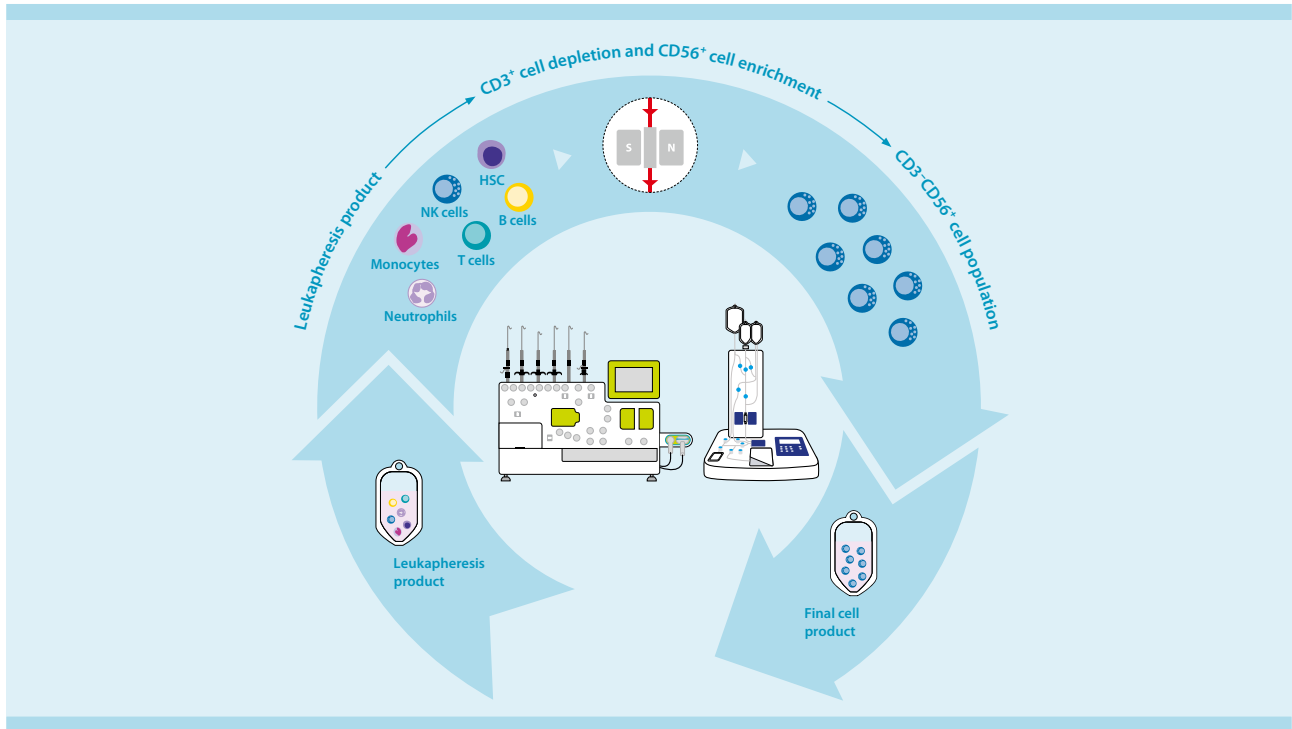
Process overview

The overview of the CliniMACS Prodigy® LP-3-56 System shows the workflow for the automated NK cell separation process. The process gives the option to do CD3⁺ cell depletion and CD56⁺ cell enrichment (case 1), CD3⁺ cell

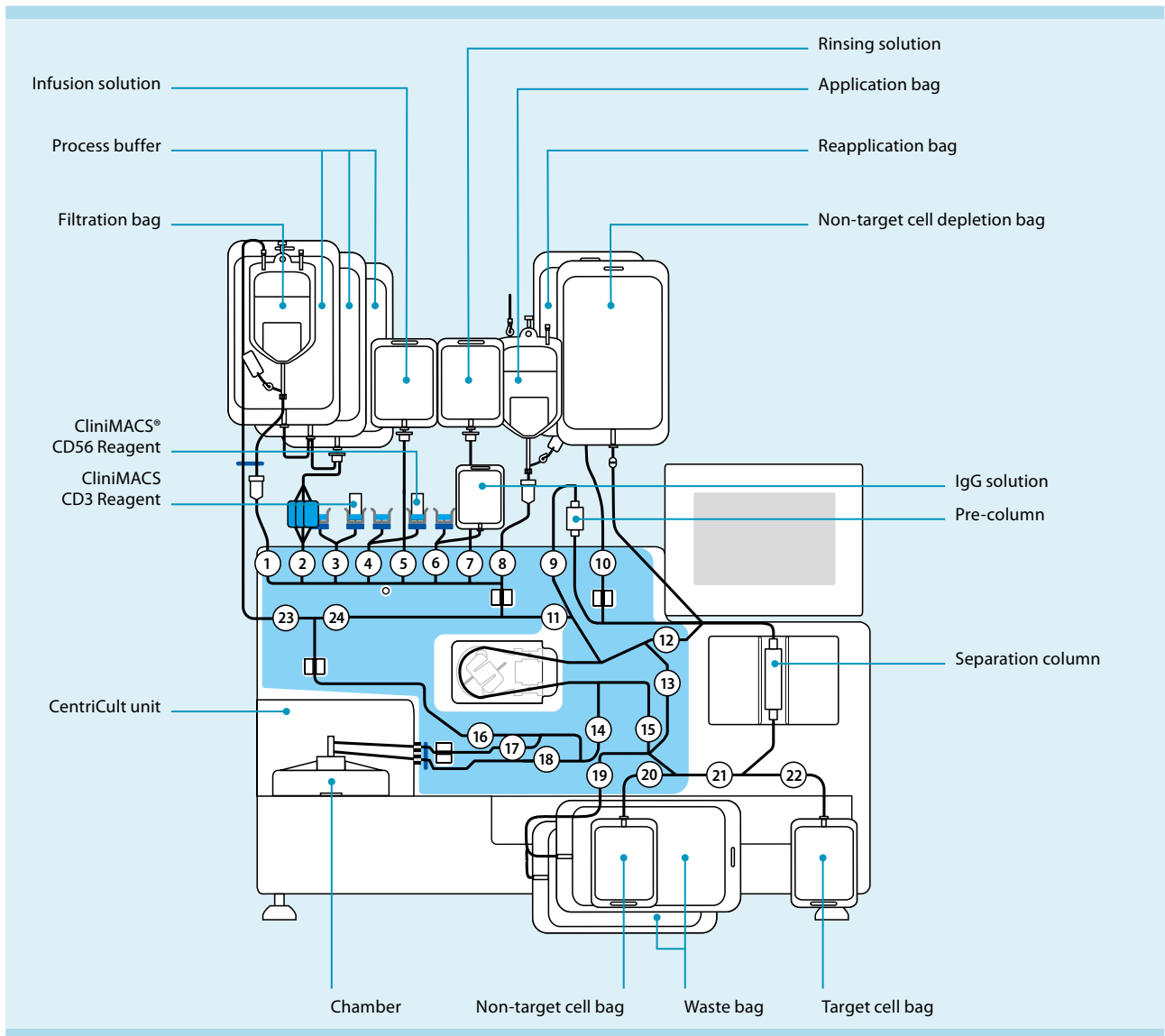
depletion only (case 2.1: CD3⁺ depleted cells are resuspended in process buffer and remain in the non-target cell bag. Case 2.2: CD3⁺ depleted cells are harvested in infusion solution) or CD56⁺ cell enrichment only (case 3).

	Case 1	Case 2.1	Case 2.2	Case 3
Pre-process	Tubing set modification, installation, and priming Process parameter input			
Depletion	Sample loading Cell washing and volume adjustment Cell labeling with IgG and CliniMACS® CD3 Reagent Cell washing Depletion			
Rinsing	Chamber and Column rinsing		Chamber and Column rinsing	
Rebuffering			Rebuffering and harvest	
Enrichment	Sample loading Cell washing and volume adjustment Cell labeling with CliniMACS CD56 Reagent Cell washing Cell filtration Enrichment			Sample loading Cell washing and volume adjustment Cell labeling with CliniMACS CD56 Reagent Cell washing Cell filtration Enrichment
Post-process	Tubing set de-installation			
Process time (approx.)	7.5–9 hours	3.5–4.5 hours	5–6 hours	4–4.5 hours

Principle of the CliniMACS Prodigy® LP-3-56 System



CliniMACS Prodigy TS 310 setup



Performance data

N=4	Starting material			
	WBC		Target cells (total CD3 ⁺ CD56 ⁺)	
	Total	Viability (%)	(%)	Total
Mean	6.38×10 ⁹	91.3	8.7	5.34×10 ⁸
SD	1.09×10 ⁹	4.2	2.3	9.10×10 ⁷

N=4	Final cell product								
	WBC		Target cells (total CD3 ⁺ CD56 ⁺)			Monocytes (CD14 ⁺)		T cells (CD3 ⁺ CD14 ⁻)	B cells (CD19 ⁺ CD14 ⁻)
	Total	Viability (%)	(%)	Total	Recovery (%)	%	Total	-logP depletion	-logP depletion
Mean	2.78×10 ⁸	93.8	94.8	2.68×10 ⁸	48.3	8.6	1.72×10 ⁷	4.2	2.7
SD	9.05×10 ⁷	5.4	4.5	9.41×10 ⁷	12.1	7.4	7.63×10 ⁶	0.2	0.1

The table shows the results from four independent unmobilized apheresis products performing the combined CD3⁺ cell depletion and CD56⁺ cell enrichment process (case 1). The mean -logP depletion is 4.2 and 2.7 for T cells and B cells, respectively. High purities of CD3⁺CD56⁺ target cells were achieved with an average recovery of 48.3%. The mean viability of the final cell product was 93.8% (SD: Standard deviation).



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