

Miltenyi Biotec

Enrichment and stimulation of primary blood dendritic cell subsets

CliniMACS Prodigy® BloodDC PreDepletion and CliniMACS Prodigy BloodDC Enrichment and Culture

Application

The CliniMACS Prodigy BloodDC PreDepletion System and the CliniMACS Prodigy BloodDC Enrichment and Culture System were developed for the joint enrichment and cultivation of dendritic cell (DC) subsets from non-mobilized apheresis products. The enriched DC subsets in this process are CD304 (BDCA-4)$^+$ plasmacytoid dendritic cells (pDCs), CD1c (BDCA-1)$^+$ myeloid dendritic cells (cDC2s), and optionally CD141 (BDCA-3)$^+$ myeloid dendritic cells (cDC1s).
The two systems are designed to be used sequentially: First, the CliniMACS Prodigy BloodDC PreDepletion System performs the depletion of CD19$^+$ and CD14$^+$ cells, and the labelling of CD1c$^+$ cells with biotin. Subsequently the CliniMACS Prodigy BloodDC Enrichment and Culture System allows the enrichment of the blood DC subsets and the stimulation and antigen loading. This application sheet gives an overview of the process specifications and the required materials.
In addition, it illustrates the process workflow and the configuration of the tubing set CliniMACS Prodigy TS 320 and TS 510, and provides performance data from internal runs.

Specifications

Process name:	CliniMACS Prodigy BloodDC PreDepletion
Selection capacity:	Up to 9×10^9 total labelled cells from 40×10^9 total white blood cells (up to 4×10^9 CD14$^+$ cells and 5×10^9 CD19$^+$ cells)
Volume starting product:	50–600 mL
Final product volume:	200–1,200 mL
Total process time:	Approx. 4.5–5.5 hours
Total hands-on time:	Up to 45 min

Process name:	CliniMACS Prodigy BloodDC Enrichment and Culture
Selection capacity:	Up to 4×10^8 total labelled cells (2×10^8 CD1c$^+$ DCs, 2×10^8 CD141$^+$ DCs and 2×10^8 CD304$^+$ DCs)
Volume starting product:	50–1,200 mL
Final product elution volume:	40 mL after cell enrichment/ 190 mL after cell culture
Total process time:	For cell enrichment only, approx. 5.5 hours. For blood DC culture, typically 6–24 hours (customizable).
Total hands-on time:	Max. 2 hours including culture, activation, and harvest

Material required for CliniMACS Prodigy BloodDC PreDepletion

CliniMACS Materials	Amount required
CliniMACS Prodigy	1
CliniMACS Prodigy TS 320	1 set
CliniMACS® CD1c (BCDA-1)-Biotin	1 vial
CliniMACS CD14 Reagent/GMP MicroBeads	1 vial
CliniMACS CD19 Reagent/GMP MicroBeads	1 vial
CliniMACS PBS/EDTA Buffer (2×3 L)	1–2 units

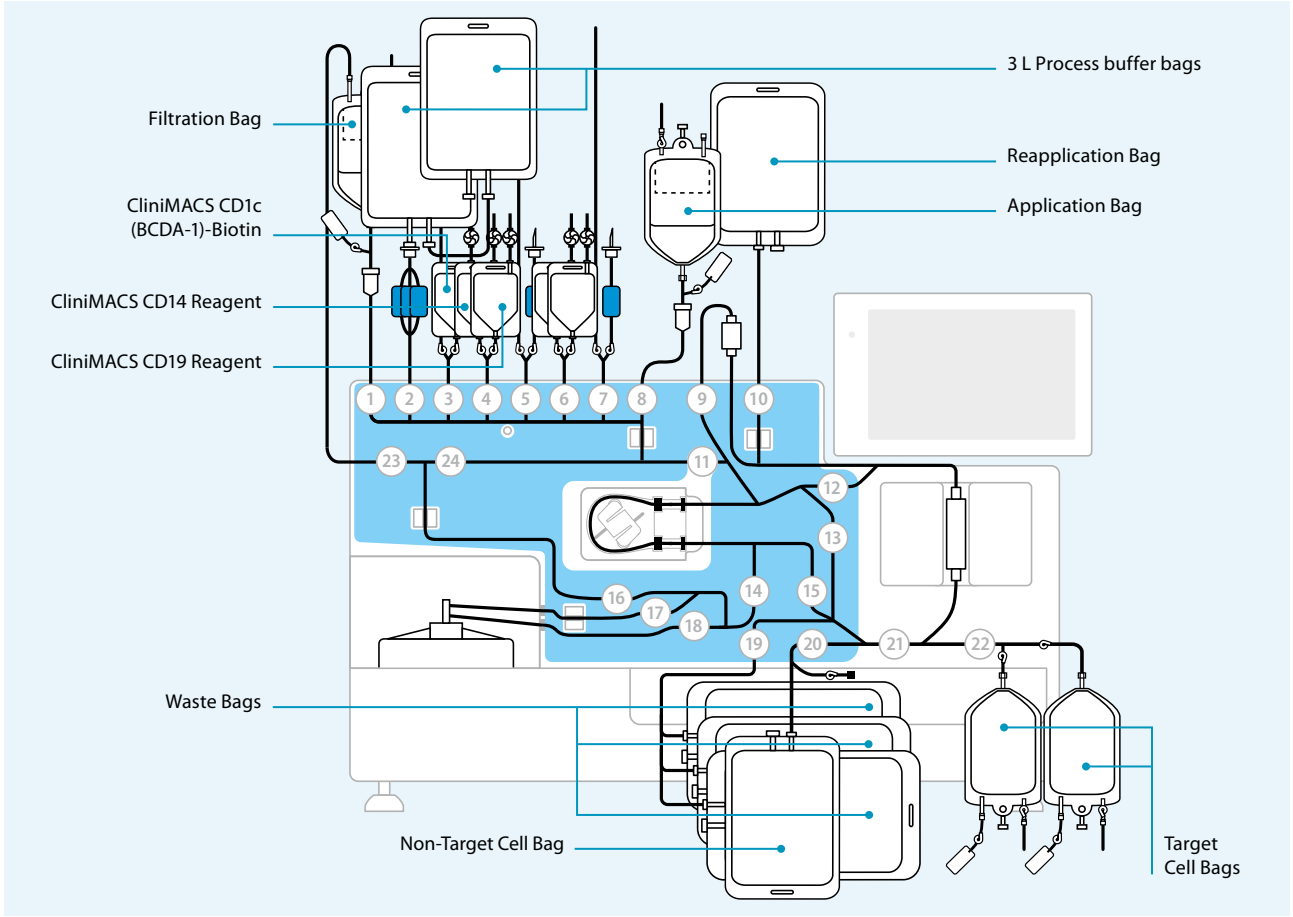
Material required for CliniMACS Prodigy BloodDC Enrichment and Culture

CliniMACS Materials	Amount required
CliniMACS Prodigy	1
CliniMACS Prodigy TS 510	1 set
CliniMACS Anti-Biotin Reagent/GMP MicroBeads	1 vial
CliniMACS CD304 (BDCA-4) Reagent/GMP MicroBeads	1 vial
CliniMACS CD141 (BDCA-3) GMP MicroBeads (only case 3 and 4)	1 vial
CliniMACS PBS/EDTA Buffer (3×1 L)	1 unit
CliniMACS PBS/EDTA Buffer (2×3 L)	1 unit
MACS® GMP Recombinant Human IL-3 (only case 1 and 2)	1 vial (25 µg/vial)
MACS GMP Recombinant Human GM-CSF (only case 1 and 2)	1 vial (25 µg/vial)
MACS GMP PepTivator® of choice (only case 1 and 2)	1 vial (60 nmol/vial)
TexMACS™ GMP Medium (only case 1 and 2)	1 L

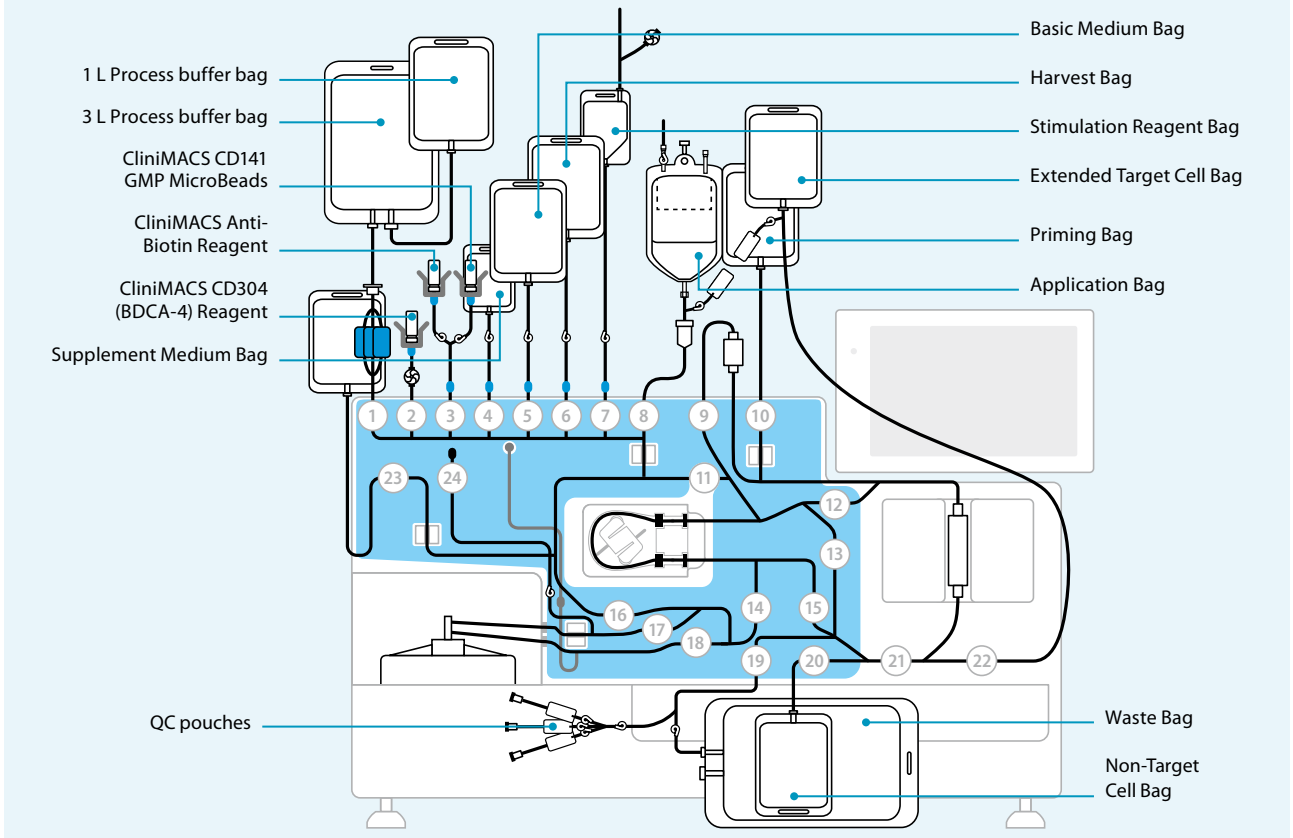
Process overview of the CliniMACS Prodigy BloodDC PreDepletion (TS 320) and the CliniMACS Prodigy BloodDC Enrichment and Culture

	CliniMACS Prodigy BloodDC PreDepletion (TS 320)	CliniMACS Prodigy BloodDC Enrichment and Culture
Pre-process	Tubing set installation and priming ▼ Connection of starting material to tubing set ▼	Tubing set installation and priming ▼ Connection of starting material to tubing set ▼
Sample preparation and labelling	Sample loading and volume adjustment ▼ Immuno-magnetic labelling of $CD14^+$ and $CD19^+$ cells and pre-labelling of $CD1c^+$ cells with biotin ▼ Cell washing ▼	Sample loading and volume adjustment ▼ Immuno-magnetic labelling of $CD1c^+$ cells via biotin, $CD304^+$ cells, and (optionally) $CD141^+$ cells ▼ Cell washing ▼
Magnetic enrichment	Magnetic enrichment ▼ Collection of target cells into target cell bag ▼	Magnetic enrichment ▼ Elution into target cell bag ▼
Pre-culture preparation	– – –	Chamber wash and transfer of cell product to chamber ▼ Addition of cell culture medium (including IL-3 and GM-CSF) and gas mix initiation ▼
Cell culture	–	Connection and addition of stimulation reagent(s) and antigen(s) to the culture ▼
Harvest and end of process	– Tubing set de-installation	Transfer of cells to harvest bag ▼ Tubing set de-installation
Process time (approx.)	**PreDepletion process: 4.5–5.5 hours**	**Enrichment step: 4–5 hours** **Cell culture step: customizable (e.g. 6–24 hours)**

CliniMACS Prodigy TS 320 setup for pre-depletion of CD14⁺ and CD19⁺ cells



CliniMACS Prodigy TS 510 setup for separation, stimulation, and antigen loading of blood DC subsets



Performance data

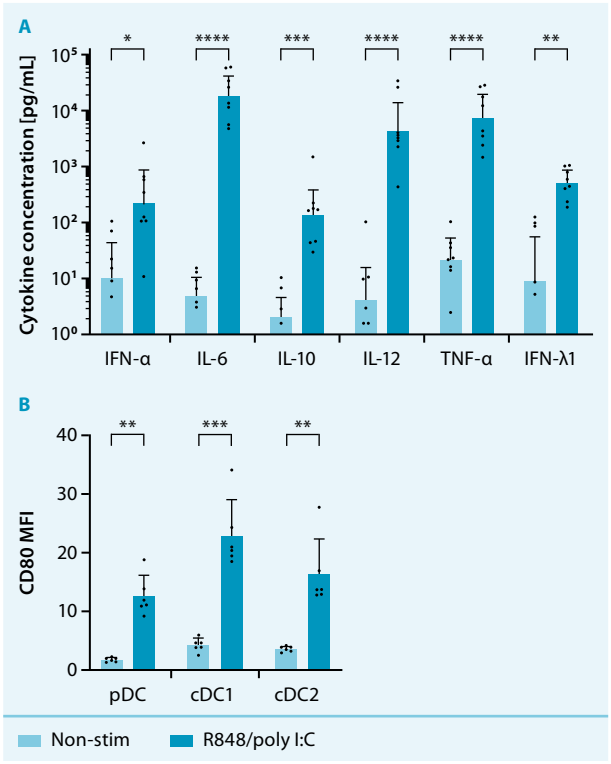


Figure 1: Cytokine secretion and activation marker expression by blood DCs enriched with CliniMACS Prodigy Enrichment and Culture System. Blood DCs were cultured in standard medium supplemented with GM-CSF and IL-3, stimulated with R848/poly(I:C), or not stimulated (non-stim) for 24 hours. Each data point represents a different blood donor. (A) Cytokine secretion of non-stimulated and stimulated blood DCs after culture. The mean with the upper limit of the 95% confidence interval is indicated (n=8). (B) Mean fluorescence intensity (MFI) of CD80 expression in pDC, cDC1, and cDC2 in non-stimulated and stimulated samples measured by flow cytometry by gating on specific subsets. The mean and the upper limit of the 95% confidence interval are indicated (n=6). Flow cytometry data was acquired by MACSQuant® Analyzer.
Statistical differences compared to non-stimulated controls were analyzed by two-way ANOVA applying the Sidak correction for multiple comparison. Significance is indicated by *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), or ****($p < 0.0001$). Data derived from our internal experiments.

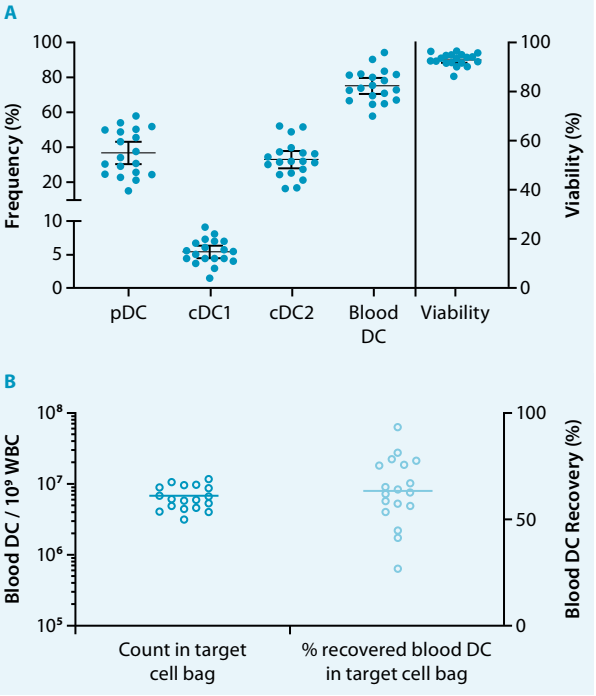


Figure 2: Purity and viability of blood DCs after enrichment with CliniMACS Prodigy PreDepletion System and CliniMACS Prodigy Enrichment and Culture System. (A) Purity is defined as frequency of each subset or all subsets together within the CD45⁺ cell population in the target cell bag after enrichment, measured by flow cytometer (values on left axis). Viability for all subsets together is expressed as frequency of viable cells within CD45⁺ cells in the target cell bag after enrichment, measured by 7-AAD staining by flow cytometer (values on right axis). (B) Count of total blood DCs in target cell bag per 1×10^9 white blood cells (WBC) after enrichment (left axis), and recovery of total blood DCs from the starting leukapheresis product (right axis) after enrichment, measured by flow cytometer. Data derived from our internal experiments.

References

1. Schwarze, J. K. *et al.* (2020) Intratumoral combinatorial administration of CD1c (BDCA-1)⁺ myeloid dendritic cells plus ipilimumab and avelumab in combination with intravenous low-dose nivolumab in patients with advanced solid tumors: a phase IB clinical trial. Vaccines 8: 670.
2. Westdorp, H. *et al.* (2019) Blood-derived dendritic cell vaccinations induce immune responses that correlate with clinical outcome in patients with chemo-naive castration-resistant prostate cancer. J. Immunother. Cancer 7: 302.

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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.
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