Miltenyi Biotec

# Manufacturing of monocyte-derived dendritic cells

## CliniMACS Prodigy® LP-14 Mo-DC

### Application

This process uses a closed, automated system to perform labeling, enrichment, differentiation, maturation, antigen loading, and rebuffering of $CD14^+$ cells from leukapheresis products to produce monocyte-derived dendritic cells (Mo-DCs).

This application sheet provides an overview of the specifications and materials required to perform the indicated process. It also outlines the workflow, configuration of the CliniMACS Prodigy Tubing Set, and relevant performance data.

### Specifications

**Monocyte enrichment**

| | |
|---|---|
| **Process name:** | LP-14 Mo-DC Process |
| **Selection capacity:** | Up to $4\times10^9$ $CD14^+$ labeled cells within $20\times10^9$ WBCs |
| **Sample volume for selection:** | 50–300 mL |
| **Process time:** | 3–3.5 hours |

**Monocyte cultivation (differentiation to Mo-DC and maturation)**

| | |
|---|---|
| **Starting cell number for cultivation:** | Up to $0.8\times10^9$ cells |
| **Expansion capacity:** | Up to $2\times10^8$ Mo-DCs in a maximum culture volume of 260 mL |
| **Final product harvest volume:** | Two harvesting modes available: 55 mL or 100 mL |
| **Process time:** | Approx. 8 days (default setting) |

### Products required

| CliniMACS® and MACS® GMP Products | Amount |
|---|---|
| CliniMACS Prodigy | 1 |
| CliniMACS Prodigy TS 510 | 1 set |
| CliniMACS CD14 Reagent (different regulatory variants available) | 1 vial |
| CliniMACS PBS/EDTA Buffer (2×3 L) | 1×3 L |
| CliniMACS PBS/EDTA Buffer (3×1 L) | 1×1 L |
| MACS GMP Recombinant Human GM-CSF | 1 vial (250 µg/vial) |
| MACS GMP Recombinant Human IL-1β | 1 vial (25 µg/vial) |
| MACS GMP Recombinant Human IL-4 | 1 vial (25 µg/vial) |
| MACS GMP Recombinant Human IL-6 | 1 vial (25 µg/vial) |
| MACS GMP Recombinant Human TNF-α | 1 vial (25 µg/vial) |
| MACS GMP PepTivators® Peptide Pools (optional) | 1 vial for $1\times10^9$ cells |

| Additional material/equipment* |
|---|
| Leukapheresis product (not older than 24 hours) |
| Human serum albumin (HSA) |
| Medium (basal, differentiation and maturation) |
| Prostaglandin $E_2$ ($PGE_2$) |
| Sterile Luer Lock syringes |
| Hypodermic needles |
| Transfer bag(s) 600 mL (module-dependent) |
| Transfer Set Coupler/Coupler (module-dependent) |
| Luer/Spike Interconnector (module-dependent) |
| 1 m extension line (optional) |

| Additional material/equipment* |
|---|
| Uninterruptible power supply |
| $CO_2$ and compressed air supply |
| Sterile tubing welder |
| Cell counter and flow cytometer |

*This protocol requires the use of components and materials that are not part of the CliniMACS Prodigy LP-14 Mo-DC System, such as human serum albumin or prostaglandine $E_2$. Only pharmaceutical-grade products approved in your country should be used. If these components are not available as registered drugs, the user must evaluate all associated risks before applying the target cell product in humans.

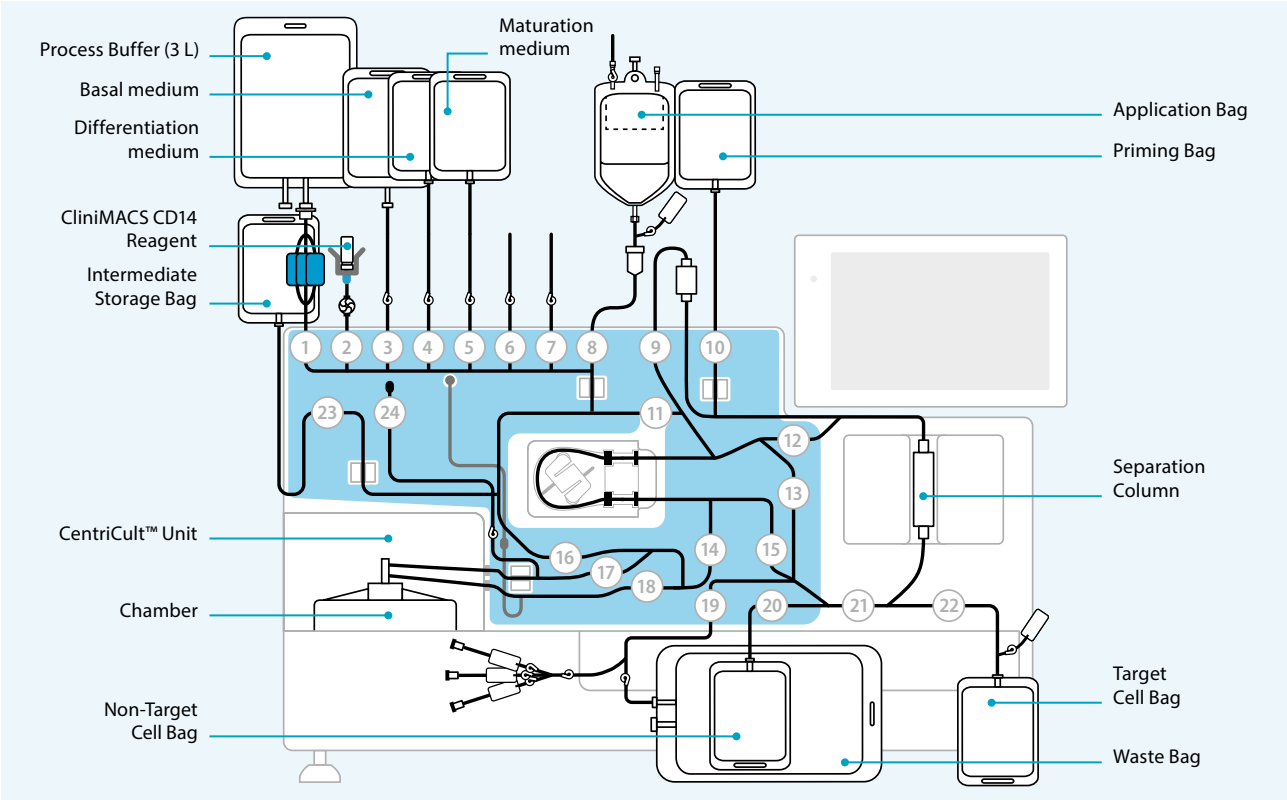
## Workflow of the CliniMACS Prodigy LP-14 Mo-DC enrichment and cultivation

| | |
|---|---|
| **Pre-process** | Process parameter input<br>▼<br>Tubing set installation and priming<br>▼ |
| **Enrichment** | Sample loading and volume adjustment<br>▼<br>Cell labelling with CliniMACS CD14 Reagent<br>▼<br>Removal of unbound beads<br>▼<br>Magnetic enrichment of $CD14^+$ cells<br>▼<br>Elution of enriched cells<br>▼ |
| **Cultivation setup** | Entry of cultivation parameters<br>▼<br>Selection of source and target port<br>▼<br>Programming of activity matrix<br>▼<br>Attachment of differentiation and maturation medium<br>▼ |
| **Cultivation** | Setup of the gas mix<br>▼<br>Start of activity matrix<br>▼ |
| **Cell harvest** | Cell washing and harvest either in 55 or 100 mL of basal medium<br>▼ |
| **Post-process** | Tubing set de-installation |

**Approx. 8 days****

** The processing time largely depends on the design and protocol of the individual workflow.

## CliniMACS Prodigy TS 510 setup for LP-14 Mo-DC Process



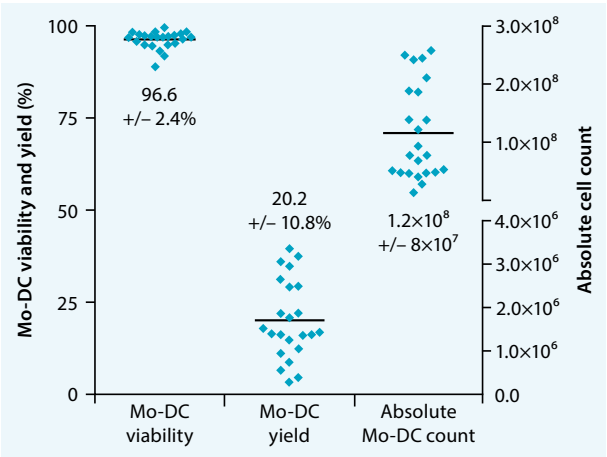




**Figure 1:** Internal data showing the viability, yield, and absolute count of monocyte-derived dendritic cells generated using the CliniMACS Prodigy LP-14 Mo-DC Process. Mature Mo-DCs (mMo-DCs) were generated with an average recovery of 20%, calculated based on the number of initially seeded monocytes. The viability of mMo-DCs was evaluated by flow cytometry, specifically by excluding $PI^+$ dead cells, with an average viability of 97%.

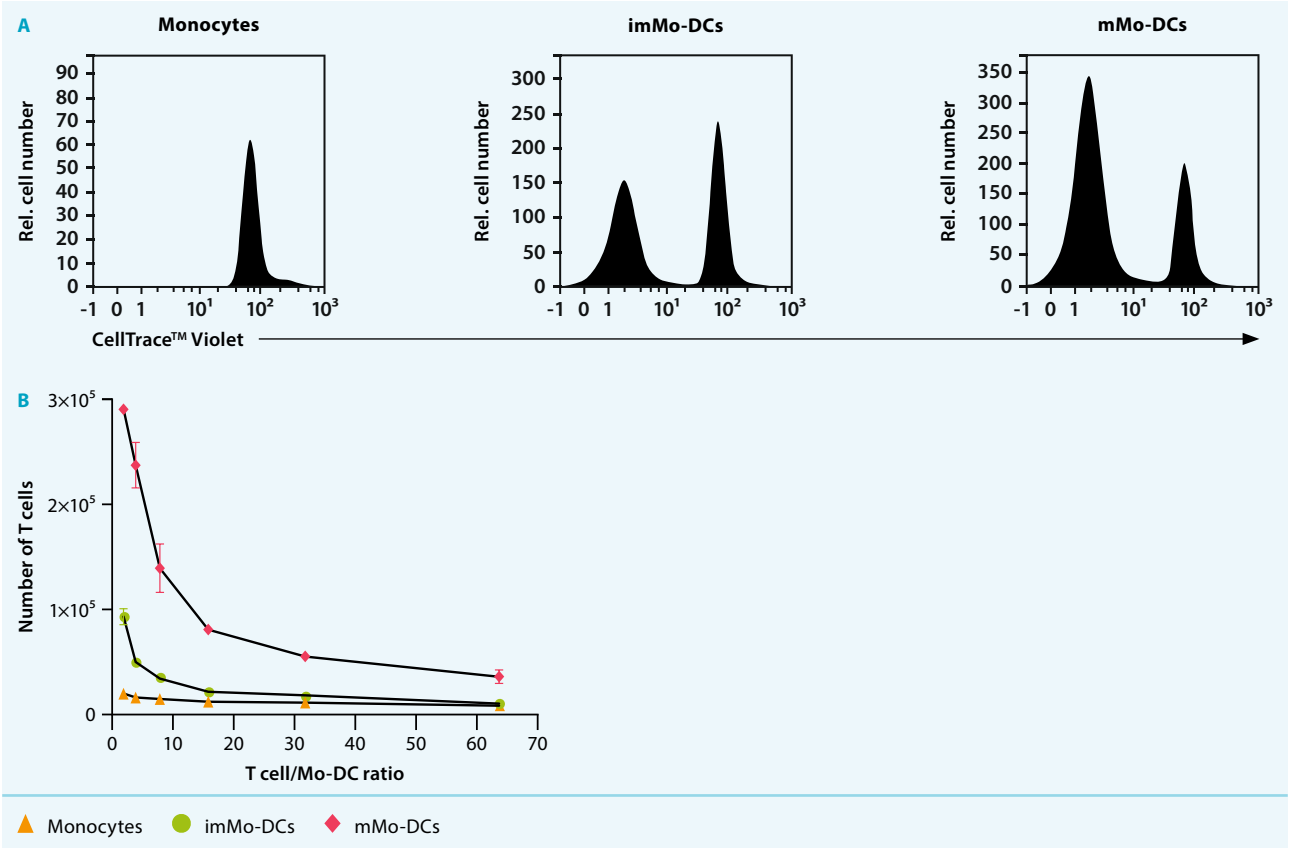



**Figure 2:** Internal data on the T cell priming capacity of monocytes and Mo-DCs isolated and cultured using the CliniMACS Prodigy. CellTrace Violet-labeled, allogeneic, naive CD45RA$^+$CD45RO$^-$ T cells were co-cultured with either monocytes or Mo-DCs. After 7 days, T cell numbers and CellTrace Violet staining intensity were analyzed (A). Unlike monocytes, both immature Mo-DCs (imMo-DCs) and mature Mo-DCs (mMo-DCs) induced T cell proliferation, as indicated by increased cell counts and reduced CellTrace Violet staining. Proliferation was highest when mMo-DCs served as antigen-presenting cells (B). In (B), the data represent the mean ± standard deviation (SD) from duplicate experiments, with error bars indicating the variability between replicates.

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