Miltenyi Biotec

Mo-DC Generation Toolbox
human

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Miltenyi Biotec

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop

1. Description

This product is for research use only.

Components	**2 mL CD14 MicroBeads, human** **400 mL Mo-DC Differentiation Medium** **2×10 µg Human TNF-α, premium grade**
Size	For 10^9 total cells, up to 10 isolations.
Product format	CD14 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide. Mo-DC Differentiation Medium contains FBS, RPMI 1640, L-glutamine, IL-4, and GM-CSF.
Storage	Store CD14 MicroBeads and Mo-DC Differentiation Medium protected from light at 2–8 °C. Do not freeze. Lyophilized Human TNF-α, premium grade should be stored at –20 °C. Avoid repeated freeze-thaw cycles. The expiration dates are indicated on the vial labels. For information about reconstitution and storage after reconstitution of the lyophilized Human TNF-α refer to chapter 2.1.

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1.1 Background information

The Mo-DC Generation Toolbox comprises reagents for magnetic cell separation of CD14^{bright} monocytes and cell culture media. Included are the reagents for the separation of monocytes by positive selection using CD14 MicroBeads, human. The Mo-DC Differentiation Medium is based on Rosewell Park Memorial Institute (RPMI) 1640 Medium with stabile glutamine and contain interleukin-4 (IL-4), and GM-CSF. Mo-DCs can easily be differentiated from monocytes with a lot-to-lot consistent standardized protocol for effective differentiation of immature and mature Mo-DCs.

Large numbers of Mo-DCs can be generated *ex vivo* from purified CD14$^+$ monocytes.[1–6] Highly pure monocytes are easily isolated from bone marrow, cord blood, peripheral blood, or peripheral blood mononuclear cells (PBMCs) by using CD14 MicroBeads, human. Excellent purity and recovery of selected monocytes provide a pure and consistent cell source. Contamination with unwanted cells (e.g. platelets) that may interfere with a controlled differentiation process is markedly reduced. The combined action of, for example, GM-CSF and IL-4 can direct monocytes to differentiate into a homogenous population of immature Mo-DCs. To produce stable mature Mo-DCs, the immature Mo-DCs can be cultured, for example, with TNF-α.

1.2 Reagent and instrument requirements

- Ficoll-Paque™ PLUS (GE Healthcare companies)
- Phosphate-buffered saline (PBS), sterile
- PE buffer: Prepare a solution containing phosphate-buffered saline (PBS) and 2 mM EDTA, pH 7.4–7.6, sterile.
- Maturation medium for cell culture: Prepare a solution of basal media (e.g. RPMI 1640), 10% fetal calf serum, 2 mM L-glutamine, and reconstituted Human TNF-α (6000 IU/mL). Human TNF-α is provided with this kit. For reconstitution of Human TNF-α refer to chapter 2.1.
- Conical tubes, sterile (15 mL and 50 mL)
- Tissue culture flask (T-75, 75 cm^2) (Becton, Dickinson and Company)
- CO_2 incubator, 37 °C, 5% CO_2 in air, and >95% humidity

- MACS Columns and MACS Separators: Positive selection or depletion of monocytes can be performed by using MS or LS Columns or by using the autoMACS® Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Penicillin-Streptomycin, liquid (Gibco®)
- (Optional) Mo-DC Differentiation Inspector (# 130-093-567)
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocols

2.1 Sample preparation

▲ Work under sterile conditions throughout the whole experiment.

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

Reconstitution of TNF-α

▲ To prepare 200 mL of maturation media you will need to use both vials of Human TNF-α provided with this kit.

Reconstitute lyophilized TNF-α with deionized sterile-filtered water to a final concentration of 0.1–1.0 mg/mL in a minimal volume of 100 µL. Further dilutions should be prepared with 0.1% bovine serum albumin (BSA) or human serum albumin (HSA) in phosphate-buffered saline. The typical biological activity is ≥ 6×10^7 IU/mg. For lot-specific activities, please contact our technical support.

Add the resuspended TNF-α to the media to reach a final concentration of 6000 IU/mL.

Upon reconstitution aliquots should be stored at –20 °C or below. Avoid

repeated freeze-thaw cycles.

2.2 Magnetic labeling using CD14 MicroBeads

▲ Work under sterile conditions.

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD14 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation of monocytes using CD14 MicroBeads (2.3).

2.3 Magnetic separation of monocytes using CD14 MicroBeads

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD14^+$ cells. For details refer to table in section 1.2.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ (Optional) For the determination of purity the use of the Mo-DC Differentiation Inspector (# 130-093-567) is recommended.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.

2. Prepare column by rinsing with the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of $CD14^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack.

   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack.

2.4 Cell culture – Mo-DC differentiation and maturation

1. Determine cell number.
2. Resuspend 10^7 cells per 10 mL of Mo-DC Differentiation Medium and mix well.
3. Plate cells in a tissue culture flask (T-75, 75 cm^2).
4. Incubate cells for 3 days at 37 °C, 5% CO_2, and >95% humidity.

5. Add 10 mL of Mo-DC Differentiation Medium to feed cells after 3 days and mix well.

   ▲ **Note:** Do not remove used Mo-DC Differentiation Medium prior to adding, simply pipette appropriate amount of fresh Mo-DC Differentiation Medium.

6. Incubate cells for 4 days at 37 °C, 5% CO_2, and >95% humidity.

7. On day 7 harvest all cells. If cells adhere release cells by adding PE buffer after removal of cell culture medium and incubate the flask for 10 minutes at 37 °C. Do not shake to release adherent cells.

8. Determine cell number of immature Mo-DCs.

   ▲ **Note:** (Optional) Use Mo-DC Differentiation Inspector for enumeration of immature Mo-DCs.

9. Resuspend immature Mo-DCs in the same volume that was used at the initiation of Mo-DC differentiation using maturation medium (chapter 2.4, step 2). For preparation of maturation medium refer to chapter 1.2.

10. Incubate cells for 3 days at 37 °C, 5% CO_2, and >95% humidity to generate mature Mo-DCs.

11. Harvest mature Mo-DCs on day 10.

▲ (Optional) Use Mo-DC Differentiation Inspector (# 130-093-567) for enumeration of mature Mo-DCs.

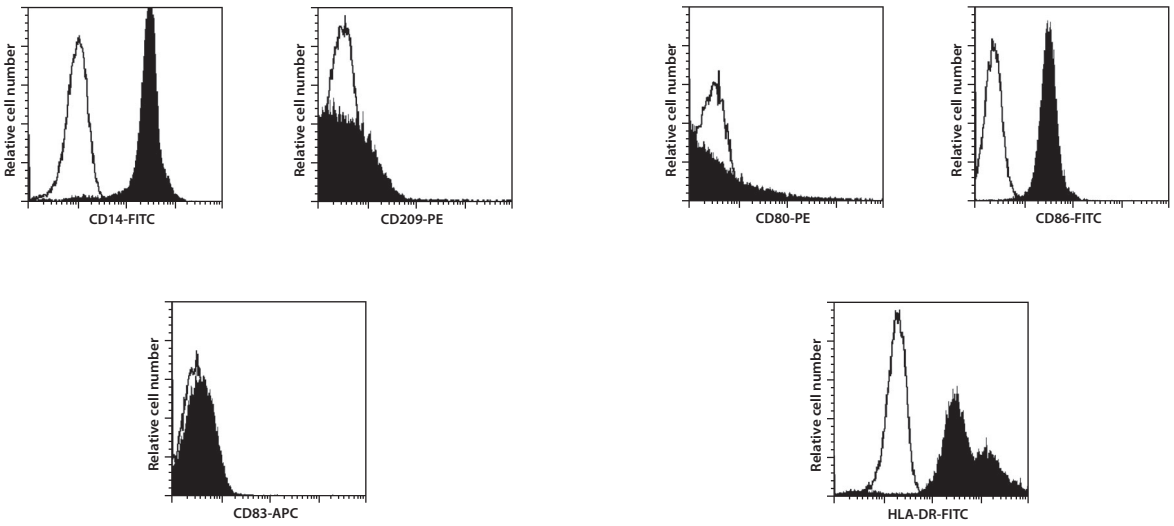
3. Example of Mo-DC generation using the Mo-DC Generation Toolbox

Monocytes were isolated from PBMCs using CD14 MicroBeads. $CD14^+$ monocytes were cultured in Mo-DC Differentiation Medium for seven days and in maturation medium for additional three days. Shown is the flow cytometric analysis of monocytes (A), immature Mo-DCs (B), and mature Mo-DCs (C) using the Mo-DC Differentiation Inspector (CD14, CD209, and CD83) and a selection of relevant markers as indicated (black histograms). Isotype control stainings are illustrated as white histograms.

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3. Example

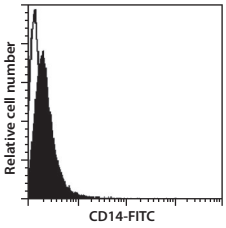
A) Isolated monocytes

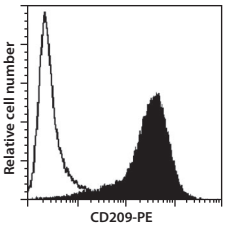


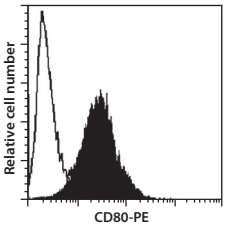
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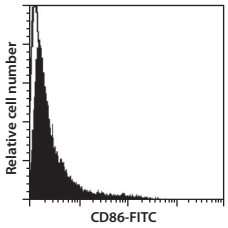
3. Example

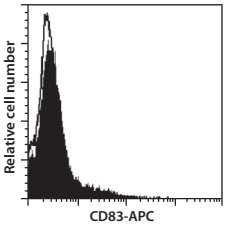
B) Immature Mo-DCs

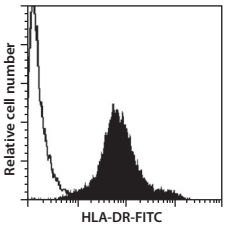








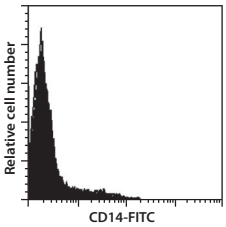


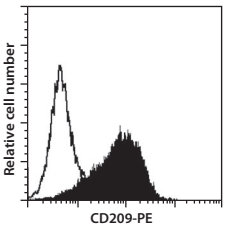


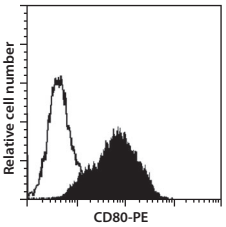
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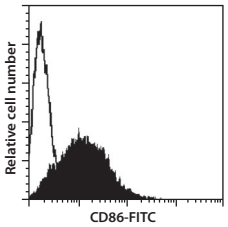
3. Example

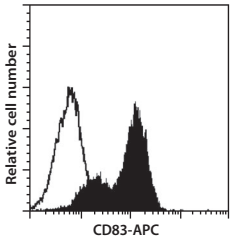
C) Mature Mo-DCs

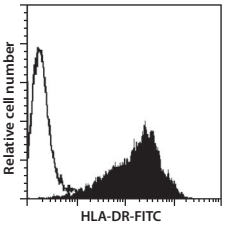












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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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