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Isolation of Classical Monocytes from PBMCs (MB Kit No. 130-117-337)

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the isolation of classical monocytes from peripheral blood mononuclear cells.

Guidelines

Magnetic labeling:

- Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labelling (but PBMC may be activated by cool, so work at RT with the cells).
- Volumes for magnetic labeling given 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 μ m nylon mesh (Pre-Separation Filters (30 μ m), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use (we use a 1mL syringe and 26-27G needle).
- The recommended incubation temperature is room temperature. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

Materials

Reagent and instrument requirements:

- **Buffer:** Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
- LS MACS Columns (Void volume: 400 μ L. Reservoir volume: 8 mL)
- MidiMACS Separator.



Safety warnings

- ! The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Before start

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.



Sample Preparation

15m

- 1 When working with anticoagulated peripheral blood or buffy coat, isolate PBMCs by density gradient centrifugation.
- 1.1 To reduce the number of thrombocytes in starting material, it is strongly recommended to perform a thrombocyte removal step. Therefore, resuspend cells in autoMACS® Rinsing Solution (# 130-091-222) and centrifuge at  200 x g, Room temperature, 00:15:00 . Thrombocytes will remain in supernatant while the cell pellet will be used for CD14+CD16– classical monocyte isolation. If necessary, repeat thrombocyte removal steps.

15m



Note

The Thrombocytes Removal Reagent can be optionally used for further removal of platelets from the sample but may result in lower recovery of monocytes.

- 1.2 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). (We have never done that).

Magnetic labeling protocol:

20m

- 2 Determine cell number.
- 3 Centrifuge cell suspension at  300 x g, 00:10:00 . Aspirate supernatant completely.
- 4 Resuspend cell pellet in  30 µL of buffer per 10⁷ total cells.
- 5 Add  10 µL FcR Blocking Reagent, human per 10⁷ total cells.
- 6 Add  10 µL of Classical Monocyte Biotin-Antibody Cocktail per 10⁷ total cells.

10m





7 **(Optional)** Add  5 μL of Thrombocyte Removal Reagent per 10^7 total cells.



8 Mix well and incubate for  00:05:00 at  Room temperature .

5m



9 Add  30 μL of buffer per 10^7 cells.



10 Add  20 μL of Anti-Biotin MicroBeads per 10^7 cells.



11 Mix well and incubate for additional  00:05:00 at  Room temperature . Proceed to magnetic separation.

5m



Magnetic Separation

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

13 Place column in the magnetic field of the MidiMACS Separator.

14 Prepare column by rinsing with the appropriate amount of buffer: LS:  3 mL .

15 Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched CD14+CD16⁻ monocytes.

16 Wash column with  3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched CD14+CD16⁻ cells, and combine with the flow-through from step 15.



**Note**

Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

- 17 **(Optional)** Remove column from the separator and place it on a suitable collection tube. Pipette  5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-monocyte cells by firmly pushing the plunger into the column.



Magnetic Separation Using LS Columns

- 18 Resuspend up to 10^8 total cells in  500 μ L of buffer.
- 18.1 When working with fresh anticoagulated blood or buffy coat, dilute before separation 1:2 with buffer. Alternatively, use the StraightFrom® Whole Blood MicroBeads or StraightFrom Buffy Coat MicroBead Kits in combination with Whole Blood Columns.
- 18.2 To remove clumps, pass cells through Pre-Separation Filters.
- 19 Apply cell suspension onto the prepared LS Column. Collect flow-through containing unlabeled cells.
- 20 Wash LS Column with  3 mL of degassed buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 19.

**Note**

Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

- 21 Remove LS Column from the separator and place it on a new collection tube.



- 22 Pipette  5 mL buffer onto the LS Column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column. 
- 23 **(Optional)** To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a second LS Column (for up to 10^8 magnetically labeled cells). Repeat the magnetic separation procedure as described in steps 19 to 22 by using a new column. 

Note

Macrophage Marker (CD11b, CD68, CD163, CD14, CD16) Antibody Panel - Human (ab254013)