Miltenyi Biotec

Macrophage Isolation Kit (Peritoneum)

mouse

Order no. 130-110-434

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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 **1 mL Macrophage (Peritoneum) Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies.

2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).

1 mL FcR Blocking Reagent,mouse

Capacity For 10^9 total cells, up to 100 separations.

Product format All components are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

Using the Macrophage Isolation Kit (Peritoneum), mouse macrophages are isolated by depletion of non-target cells. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled macrophages run through the column.

1.2 Background information

The Macrophage Isolation Kit (Peritoneum) has been developed for the isolation of untouched large and small peritoneal macrophages from mouse peritoneal cavity. Isolation of highly pure macrophages is achieved by depletion of magnetically labeled non-target cells, i.e., T cells, B cells, NK cells, dendritic cells, erythroid cells, and granulocytes.

1.3 Applications

- Isolation of mouse macrophages from peritoneal cavity for *in vitro* analysis, differentiation studies, co-culture, or adoptive transfer experiments.
- Isolation of macrophages from peritoneal cavity of thioglycolate-stimulated mice

1.4 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.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

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) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-F4/80-FITC and Anti-MHC Class II (I-ab)-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) 7-AAD for flow cytometric exclusion of dead cells.
- (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.

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2. Protocol

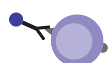
2.1 Sample preparation

Preparation of a single-cell suspension from mouse peritoneum

1. Cut the outer skin of the peritoneum using scissors and forceps.
2. Gently pull outer skin back to expose the inner skin lining the peritoneal cavity.
3. Inject 5 mL of buffer into the peritoneal cavity using a syringe and a 27G needle. Be careful not to puncture any organs.
4. After injection, gently massage the peritoneum.
5. Collect fluid containing macrophages using a 5 mL syringe. Avoid clogging of the needle by fat tissue or organs.
6. Collect as much of the injected buffer as possible.
7. Optionally repeat preparation steps. Collect cell suspension in tubes on ice.
8. Proceed to magnetic labeling (2.2).

▲ 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).

▲ Macrophages are highly adherent cells. For highest recovery prime tubes with protein-containing buffer before use.



2.2 Magnetic labeling

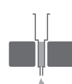
▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ 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 36 µL of buffer and add 4 µL of FcR Blocking Reagent per 10^7 total cells.
4. Add 10 µL of Macrophage (Peritoneum) Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 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 cell pellet in 80 µL of buffer per 10^7 cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of cells. For details refer to table in section 1.4.

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

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, representing the enriched macrophages.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched macrophages, and combine with the flow-through from step 3.
   MS: 3×500 µL LS: 3×3 mL
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled non-macrophages by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL

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Magnetic separation with the autoMACS® Pro Separator

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

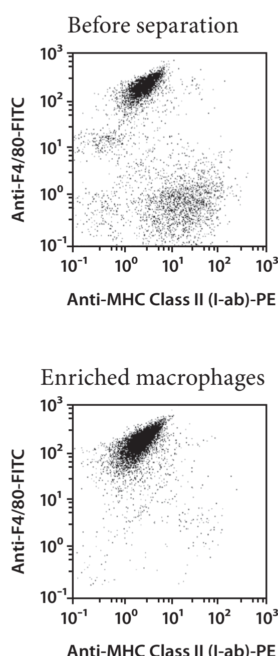
▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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 and collection tubes into the Chill Rack.
3. For a standard separation choose the following program:

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched macrophages.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-macrophages.

3. Example of a separation using the Macrophage Isolation Kit (Peritoneum)

Macrophages (F4/80+ cells) were isolated from peritoneal cell suspension by using the Macrophage Isolation Kit (Peritoneum), an MS Column, and a MiniMACS™ Separator. The cells were fluorescently stained with Anti-F4/80-FITC and Anti-MHC Class II (I-ab)-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence.



4. References

1. Rosas, M. *et al.* (2014) The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. Science 344(6184): 645–648.
2. Ghosn, E. E. B. *et al.* (2009) Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. PNAS U.S.A. 107(6): 2568–2573.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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