Miltenyi Biotec

Anti-F4/80 MicroBeads UltraPure

mouse

Order no. 130-110-443

Contents

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 Anti-F4/80 MicroBeads UltraPure, mouse: UltraPure MicroBeads conjugated to monoclonal anti-mouse F4/80 antibodies (isotype: human IgG1).
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	Anti-F4/80 MicroBeads UltraPure 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

First, the F4/80$^+$ cells are magnetically labeled with Anti-F4/80 MicroBeads UltraPure. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled F4/80$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of F4/80$^+$ cells. After removing the column from the magnetic field, the magnetically retained F4/80$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the F4/80$^+$ cells must be separated over a second column.

1.2 Background information

F4/80 is a member of epidermal growth factor (EGF)-transmembrane 7 (TM7) family and consists of a seven-span transmembrane molecule with a large extracellular, multiple EGF module–containing, domain. F4/80 is considered as one of the most specific cell-surface markers for murine macrophages. Constitutive and high expression of F4/80 is found on most resident tissue macrophages, including spleen, microglia in the brain, Kupffer's cells in the liver, and Langerhans cells in the skin. In addition, the expression of F4/80 can also be regulated depending on the physiological status of the cell.

The Anti-F4/80 MicroBeads UltraPure have been optimized for macrophages enrichment from mouse spleen and some tumors, e.g., Lewis lung carcinoma. For more information contact Miltenyi Biotec Technical Support.

1.3 Applications

- Positive selection or depletion of cells expressing mouse F4/80 antigen from mouse spleen and some tumors.

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.
- MACS Columns and MACS Separators: F4/80$^+$ cells can be enriched by using MS or LS Columns. Positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
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., CD45-VioBlue®, CD11b-APC, and Anti-F4/80-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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- (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. Protocol

2.1 Sample preparation

When working with lymphoid organs or non lymphoid-tissues, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.com/protocols.

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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of Anti-F4/80 MicroBeads UltraPure per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the dark 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 (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 F4/80$^+$ cells. For details refer to the 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.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through 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. 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. To increase the purity of F4/80$^+$ cells, the eluted fraction must 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 the following program:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

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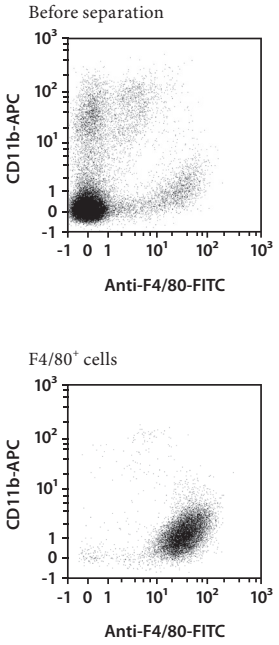
3. Example of a separation using the Anti-F4/80 MicroBeads UltraPure

F4/80$^+$ cells were isolated from spleen single-cell suspension using Anti-F4/80 MicroBeads UltraPure, two MS Columns, and an OctoMACS™ Separator. The cells were fluorescently stained with CD45-VioBlue®, CD11b-APC, and Anti-F4/80-FITC 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. Austyn, J. M. and Gordon, S. (1981) F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. 11: 805–815.
2. Wynn, T. A. *et al.* (2013) Macrophage biology in development, homeostasis and disease. Nature 496: 445.

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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