Anti-Siglec-F MicroBeads
mouse

Order no. 130-118-513

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-Siglec-F MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse Siglec-F antibodies (isotype: rat IgG1).
Capacity	For 10^9 total cells, up to 100 separations, when working with mouse peripheral blood. For 2×10^9 total cells, up to 200 separations, when working with mouse lung.
Product format	Anti-Siglec-F MicroBeads 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 Siglec-F$^+$ cells are magnetically labeled with Anti-Siglec-F MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled Siglec-F$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of Siglec-F$^+$ cells. After removing the column from the magnetic field, the magnetically retained Siglec-F$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the Siglec-F$^+$ cells must be separated over a second column.

1.2 Background information

Siglec-F, also known as Siglec-5, is a member of the sialic acid-binding immunoglobulin (Ig)-like lectins (Siglecs). The extracellular Ig domain binds specifically carbohydrate structures containing sialic acid residues. Siglec-F has, like most Siglecs, an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic domain and is discussed to play a role in eosinophil accumulation and survival. It is expressed in a tissue dependent manner on most eosinophils, but also found on alveolar macrophages and some neutrophils.

1.3 Applications

- Positive selection or depletion of cells expressing mouse Siglec-F antigen from mouse peripheral blood (eosinophils, minor fraction of neutrophils) or mouse lung single-cell suspensions (eosinophils and alveolar macrophages).

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Siglec-F$^+$ 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., Anti-Siglec-F-APC and CD11c-PE-Vio® 770. 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.

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- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated mouse peripheral blood, remove red blood cells before magnetic labeling.

When working with mouse lung, prepare a single-cell suspension using the Lung Dissociation Kit, mouse (# 130-095-927) in combination with the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.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).

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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) 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. When working with single-cell suspension from mouse lung:
   Resuspend cell pellet in 90 µL of buffer per 10^7 total cells. Add 10 µL of Anti-Siglec-F MicroBeads per 10^7 total cells.

   When working with mouse peripheral blood:
   Resuspend cell pellet in 80 µL of buffer per 10^7 total cells. Add 20 µL of Anti-Siglec-F MicroBeads per 10^7 total cells.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. 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 Siglec-F$^+$ 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 Siglec-F$^+$ 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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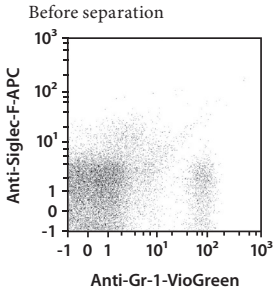
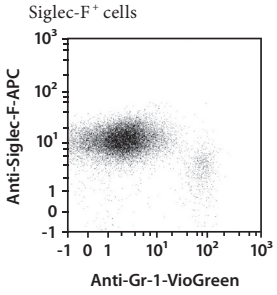
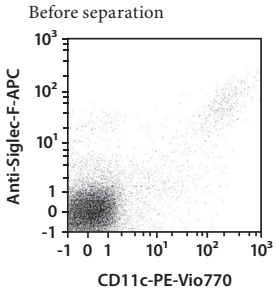
3. Example of a separation using Anti-Siglec-F MicroBeads

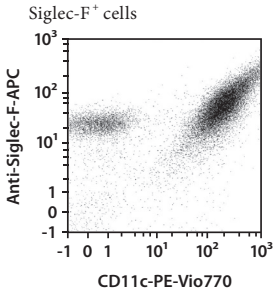
Siglec-F$^+$ eosinophils were isolated from mouse peripheral blood using Anti-Siglec-F MicroBeads, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with Anti-Siglec-F-APC and Anti-Gr-1-VioGreen™ and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





Siglec-F$^+$ alveolar macrophages and eosinophils were isolated from mouse lung single-cell suspension using Anti-Siglec-F MicroBeads, two MS Columns, and a MiniMACS Separator. Cells were fluorescently stained with Anti-Siglec-F-APC and CD11c-PE-Vio® 770 and analyzed by flow cytometry using the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





4. References

1. Zhang, M. *et al.* (2007) Defining the *in vivo* function of Siglec-F, a CD33-related Siglec expressed on mouse eosinophils. Blood 109(10): 4280–4287.
2. Bochner, B. (2009) Siglec-8 on human eosinophils and mast cells, and Siglec-F on murine eosinophils, are functionally related inhibitory receptors. Clin. Exp. Allergy 39 (3): 317–324.
3. Guo, J. P. *et al.* (2011) Characterization of expression of glycan ligands for Siglec-F in normal mouse lungs. Am. J. Respir. Cell Mol. Biol. 44(2): 238–243.

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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