Miltenyi Biotec

CD115 MicroBead Kit
mouse

Order no. 130-096-354

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1. Description

Components

1 mL CD115-Biotin, mouse:
monoclonal CD115 antibody conjugated to biotin (isotype: rat IgG2a).

1 mL FcR Blocking Reagent, mouse

2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to monoclonal anti-biotin antibodies.

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

Product format All reagents 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 CD115$^+$ cells are indirectly magnetically labeled with CD115-Biotin antibodies and Anti-Biotin 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 CD115$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD115$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD115$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD115$^+$ cells must be separated over a second column.

1.2 Background information

The CD115 MicroBead Kit has been developed for positive selection of mouse CD115$^+$ cells from lymphoid tissues. CD115 is expressed on monocytes, macrophages, osteoclasts as well as common dendritic cell precursors (CDP) and macrophage/dendritic cell precursors (MDP).

1.3 Applications

- Isolation of mouse CD115$^+$ cells for *in vitro* analysis, differentiation studies, co-culture, or adoptive transfer experiments.
- Isolation of CD115$^+$ monocytes from bone marrow.

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: CD115$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD115 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS 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
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **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 CD115 antibodies for flow cytometric analysis, e.g., CD115-PE (# 130-096-308) and Anti-Ly-6C-APC (# 130-093-136). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 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 (# 130-041-407) to remove cell clumps.

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140-003-262.02

2. Protocol

2.1 Sample preparation

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

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).



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 FcR Blocking Reagent per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of CD115-Biotin per 10^7 total cells.
7. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
10. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
11. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
12. (Optional) Add staining antibodies, e.g., 10 µL of CD115-PE (# 130-096-308) after 10 minutes of this incubation period and continue incubation for the remaining 5 minutes.
13. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
15. 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 $CD115^+$ cells. For details refer to the table in section 1.4.

▲ To achieve highest purities, perform two consecutive column runs.

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

Magnetic separation with MS or LS Columns

1. Place MS or LS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective 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 only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a 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 $CD115^+$ cells, enrich the eluted fraction over a second MS or LS Column, respectively. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

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

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

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

Magnetic separation with the autoMACS® Separator

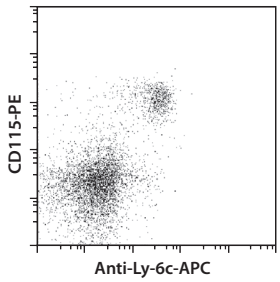
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:

   Positive selection: Posseld2
   Collect positive fraction from port pos2.

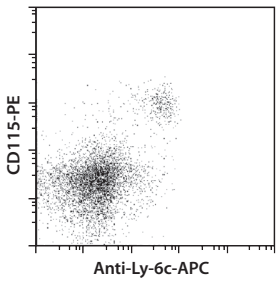
3. Example of a separation using the CD115 MicroBead Kit

$CD115^+$ cells were isolated from C57BL/6 bone marrow cells using the CD115 MicroBead Kit, two MS Columns and an OctoMACS Separator. Cells were fluorescently stained with CD115-PE (# 130-096-308) and Anti-Ly-6C-APC (# 130-093-136). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

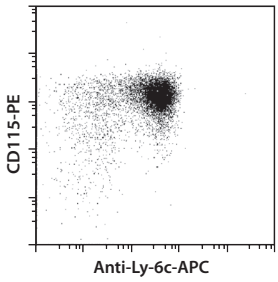
Before separation



$CD115^-$ cells



$CD115^+$ cells



4. References

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3. Sunderkötter, C. *et al.* (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J. Immunol. 172: 4410–4417.
4. Auffray, C. *et al.* (2009) CX_3CR1^+ $CD115^+$ $CD135^+$ common macrophage/DC precursors and the role of CX_3CR1 in their response to inflammation. J. Exp. Med. 206: 595–606.

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.

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.

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