Miltenyi Biotec

Anti-MHC Class II MicroBeads
mouse

Order no. 130-052-401

Index

1. Description

Components	2 mL Anti-MHC Class II MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse MHC Class II (isotype: rat IgG2b) antibody.
Size	For 2×10^9 total cells, up to 200 separations.
Product format	Anti-MHC Class II MicroBeads are supplied as a suspension 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 MACS® Separation

First, MHC Class II$^+$ cells are magnetically labeled with Anti-MHC Class II MicroBeads. Then, the cell suspension is loaded onto a column which is placed in the magnetic field of a MACS® Separator. The magnetically labeled MHC Class II$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of MHC Class II$^+$ cells. After removal of the column from the magnetic field, the magnetically retained MHC Class II$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Anti-MHC Class II MicroBeads were developed for separation of cells based on the expression of mouse MHC Class II molecules. MHC Class II molecules are expressed on B cells, monocytes, macrophages, dendritic cells, hematopoietic progenitor cells and thymic epithelial cells.

Example applications

- Positive selection or depletion of MHC Class II$^+$ cells from spleen, lymph nodes, thymus, bone marrow, peripheral blood, body fluids or nonhematopoietic tissue.
- Isolation of untouched T cells from lymphoid tissue or spleen by depletion of MHC Class II expressing cells.

1.3 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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: MHC Class II$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). Anti-MHC Class II MicroBeads can be used for depletion of MHC Class II$^+$ cells on LD, CS or D Columns. Cells which strongly express the MHC Class II antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated Anti-MHC Class II antibodies, e.g., Anti-MHC Class II-FITC (# 130-081-601), Anti-MHC Class II-PE (# 130-091-368) or Anti-MHC Class II-APC (# 130-091-806).
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

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2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs, non-lymphoid tissue or peripheral blood using standard methods. For details see General Protocols in the User Manuals or visit www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells we recommend to remove dead cells by density gradient centrifugation or using 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

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-MHC Class II MicroBeads per 10^7 total cells.
5. Mix well and refrigerate for 15 minutes (4–8 °C).

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. (Optional) Add a fluorochrome-conjugated Anti-MHC Class II antibody, according to manufacturer's recommendation, and refrigerate for 5 minutes (4–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend cell pellet in 500 µL of buffer for up to 1.25×10^8 cells.
9. 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 MHC Class II^+ cells. For details see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   Collect total effluent; this is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an 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

   ▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells over a new, freshly prepared column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent. This is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation, refer to the D Column data sheet.

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Magnetic separation with the autoMACS™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose one of the following separation programs:

   Positive selection: "Possel"

   Depletion: "Depletes"

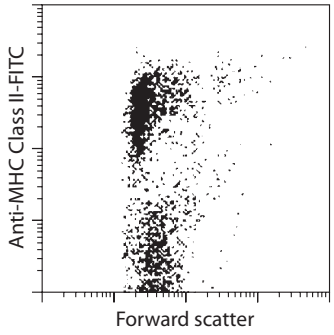
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Possel", collect positive fraction from outlet port pos1. This is the purified MHC Class II$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction from outlet port neg1. This is the MHC Class II$^-$ cell fraction.

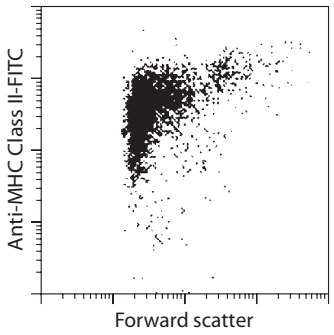
3. Examples of separations using Anti-MHC Class II MicroBeads

A: MHC Class II$^+$ cells were isolated from a spleen cell suspension using Anti-MHC Class II MicroBeads, a MiniMACS™ Separator and an MS Column. The cells are fluorescently stained with Anti-MHC Class II-FITC (# 130-081-601). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Spleen cells before separation

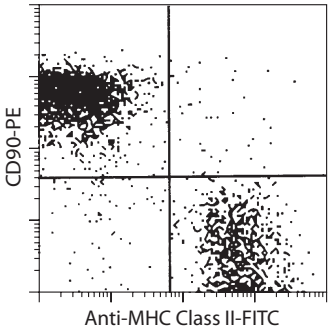


MHC Class II$^+$ cells

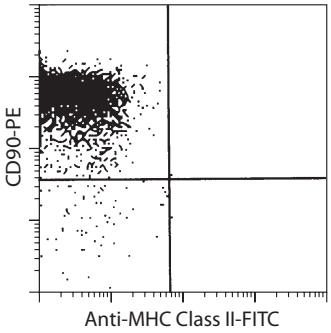


B: Isolation of untouched T cells from mouse lymph nodes by depletion of MHC class II expressing cells using Anti-MHC Class II MicroBeads, a MiniMACS Separator and an MS Column. Cells are fluorescently stained with Anti-MHC Class II-FITC (# 130-081-601) and CD90-PE (# 130-091-601). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Lymph node cells before separation



Enrichment of untouched T cells



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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