



Magnetic cell sorting of rat leukocytes

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1. Description

Component	2 ml Anti-Rat MHC Class II (OX6) MicroBeads for 10 ⁹ total cells
Specificity	Non-polymorphic epitope of rat MHC Class II molecules
Clone	OX6
Isotype	Mouse IgG1
Product format	The product is supplied as suspension containing 0.1 % gelatine and 0.05 % sodium azide.
Storage	Store protected from light at 4°C. Do not freeze. The expiry date is indicated on the vial label.

1.1 Principle of a MACS separation

For MACS separation, cells are magnetically labeled with Anti-Rat MHC Class II (OX6) MicroBeads and passed through a separation column which is placed in the magnetic field of a MACS Separator. The magnetically labeled MHC II⁺ cells are retained in the column while the unlabeled MHC II⁻ cells run through. After removal of the column from the magnetic field, the magnetically retained MHC II⁺ cells can be eluted as magnetically labeled cell fraction.

1.2 Reagent and instrument requirement

- Buffer (degassed): phosphate buffered saline pH 7.2, supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Keep buffer cold (4°-8°C).
 - ▲ **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 gelatine, human serum albumin, human serum, bovine serum.
 - Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Pre-Separation Filter (# 130-041-407)

Anti-Rat MHC Class II (OX6) MicroBeads

2 ml Anti-Rat MHC Class II (OX6) MicroBeads

For 10⁹ total cells

Order No. 130-090-759

- Columns and separators:

Column	max. number of labeled cells	max. number of total cells	Separator
Positive selection and depletion			
MS	10 ⁷	2 x 10 ⁸	MiniMACS, OctoMACS; with Column Adapter: VarioMACS, SuperMACS
LS	10 ⁸	2 x 10 ⁹	MidiMACS with Column Adapter: VarioMACS, SuperMACS
XS	10 ⁹	2 x 10 ¹⁰	SuperMACS
Depletion			
LD	10 ⁸	5 x 10 ⁸	MidiMACS with Column Adapter: VarioMACS, SuperMACS
CS	2 x 10 ⁸		SuperMACS, VarioMACS
D	10 ⁹		SuperMACS
Positive selection and depletion			
autoMACS	2 x 10 ⁸	4 x 10 ⁹	autoMACS

1.3 Background and product applications

Anti-Rat MHC Class II (OX6) MicroBeads are developed for the positive selection or depletion of rat cells expressing a non-polymorphic epitope¹ of major histocompatibility complex class II molecules². MHC Class II molecules are expressed by all rat strains on APC, i.e. B cells, dendritic cells³, and monocytes/macrophages. Epithelial, endothelial⁴ and microglia⁵ cells as well as T and NK cells⁶ may also express MHC II molecules under certain circumstances. Rat T cells can also acquire MHC Class II molecules by absorption or vesicle transfer.⁷

Rat MHC Class II⁺ cells isolated using MACS can be used for studies on antigen-presentation and T cell stimulation in rat, e.g. for mixed leukocyte reactions. Alternatively, purified MHC Class II⁺ cells can be used to delineate cell differentiation or activation patterns. Apart from enrichment of APC, Anti-Rat MHC Class II (OX6) MicroBeads can also be used to enrich untouched T cells from lymph nodes by depletion of MHC Class II⁺ non-T cells. The monoclonal antibody OX6 has already been used for magnetic separation by MACS using Rat-Anti-Mouse IgG1 MicroBeads.³

2. Protocol

2.1 Sample preparation

- ▲ Prepare a single cell suspension from lymphoid or non-lymphoid tissue by standard preparation method (for details see General Protocols in User Manuals or visit our website www.miltenyibiotec.com).

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▲ For a proper performance it is important to obtain a single cell suspension before magnetic separation. To remove cell clumps which may clog the columns pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407).

▲ Dead cells may bind nonspecifically to MACS MicroBeads. To remove dead cells, we recommend density gradient centrifugation using Ficoll-Paque™ or using the Dead Cell Removal Kit (# 130-090-101).



2.2 Magnetic labeling

▲ Avoid capping of antibodies on the cell surface during labeling and avoid nonspecific cell labeling. Therefore work fast, keep cells cold, use cold solutions only. Working on ice requires increased incubation times for MACS MicroBeads. Incubate in refrigerator at 4°–8°C.

1. Wash cells by adding 10–20x labeling volume of buffer, centrifuge at 300xg for 10 minutes.
2. Pipette off the supernatant completely.
3. Resuspend cell pellet in 80 µl of buffer per 10⁷ total cells.

▲ **Note:** When working with lower cell numbers, use same volumes. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e. g. for 2 x 10⁷ total cells use twice the volume of all indicated reagent volumes and total volumes).
4. Add 20 µl of Anti-Rat MHC Class II (OX6) MicroBeads per 10⁷ total cells.

▲ **Note:** Increased Anti-Rat MHC Class II (OX6) MicroBead concentrations may lead to unspecific binding to non-target cells and may also impair antibody fluorochrome staining when using an antibody of the same specificity.
5. Mix well and incubate for 15 minutes at 4°–8°C.

▲ **Note:** Higher temperatures and longer incubation times for magnetic labeling and staining may lead to unspecific cell labeling.
6. (Optional) Add fluorochrome-conjugated antibody at the titer recommended by the manufacturer and incubate for additional 5–10 minutes to evaluate the efficiency of the magnetic separation by flow cytometry or fluorescence microscopy.
7. Wash cells by adding 10–20x the labeling volume of buffer, centrifuge at 300xg for 10 minutes.
8. Pipette off the supernatant.
9. Resuspend cell pellet in 500 µl buffer for up to 10⁸ total cells.

▲ **Note:** Larger numbers of cells in the starting sample require a larger buffer volume when applying cells onto separation column. Use a maximum cell number of 10⁸ cells per 500 µl of buffer.
10. Proceed to magnetic separation.

2.3 Magnetic separation



Magnetic separation with MS and LS Columns

1. Choose a MS Column (up to 10⁷ magnetically labeled cells and up to 2 x 10⁸ total cells) or a LS Column (up to 10⁸ magnetically labeled cells and up to 2 x 10⁹ total cells). Place the column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:

MS: 500 µl	LS: 3 ml.
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3. Apply cell suspension in suitable amount of buffer onto the column:

MS: 500–1000 µl	LS: 1–10 ml.
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 Allow the cells to pass through and collect effluent as fraction with unlabeled cells.
4. Wash with appropriate amount of buffer:

MS: 3 x 500 µl	LS: 3 x 3 ml.
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 Allow the cells to pass through and collect effluent as fraction with unlabeled cells.
5. Remove column from separator and place column on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column:

MS: 1 ml	LS: 5 ml.
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7. Firmly flush out magnetically labeled cells using the plunger supplied with the column.

▲ **Note:** To increase purity of the magnetically labeled cell fraction, it can be passed over a second, freshly prepared column.



Depletion with LD Columns

1. Choose a LD Column of up to 10⁸ magnetically labeled cells and up to 5x10⁸ total cells. Place LD Column in the magnetic field of a suitable MACS Separator (see "LD Column data sheet").
2. Prepare column by rinsing with 2 ml of buffer.
3. Apply cell suspension in 500 µl buffer per 1.25x10⁸ total cells on top of the column.
4. Collect the unlabeled cells which pass through and wash with 2x1 ml of buffer. Collect total effluent as unlabeled fraction.



Magnetic Separation with XS Columns

Magnetic separation for up to 10⁹ positive cells and up to 2x10¹⁰ total cells can be achieved by using XS Columns. Place the column in a SuperMACS. For details on the column assembly and the separation, refer to the "XS Column data sheet".



Depletion with CS Columns

1. Assemble the CS Column and place it in the magnetic field of a suitable MACS Separator (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 (see "CS Column data sheet").
3. Apply cell suspension in 500 µl buffer per 10⁸ total cells on top of the column.
4. Collect the unlabeled cells which pass through and wash with 30 ml buffer from top. Collect total effluent as unlabeled fraction.



Depletion with D Columns

Magnetic separation for up to 10⁹ magnetically labeled cells can be achieved by using D Columns. Place the column in a SuperMACS. For details on the column assembly and the separation, refer to the "D Column data sheet".

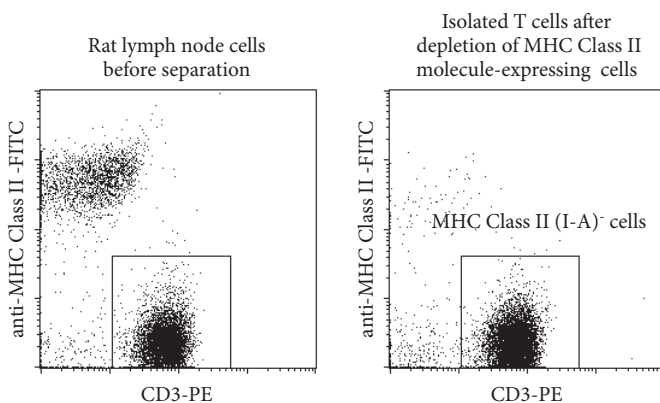


Magnetic separation with autoMACS

Positive selection or depletion of up to 2×10^8 magnetically labeled cells from up to 4×10^9 total cells, can be achieved by using autoMACS. For the selection of the appropriate program please refer to the autoMACS User Manual.

3. Example for a separation of rat lymph node cells using Anti-Rat MHC Class II (OX6) MicroBeads

Depletion of MHC II⁺ cells from a single cell suspension of rat lymph nodes using Anti-Rat MHC Class II (OX6) MicroBeads, MiniMACS and a MS Column. Cells are stained with anti-MHC Class II-FITC (clone OX6) and CD3-PE (clone G4.18). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

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Warning

Reagents contain sodium azide. Sodium azide yields hydrazoic acid under acid conditions, which is extremely toxic. Azide compounds should be diluted with running water before discarded. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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