Miltenyi Biotec

CD45R (B220) MicroBeads
mouse

Order No. 130-049-501

Index

1. Description

Components	2 mL CD45R (B220) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD45R (B220; isotype: rat IgG2a; clone:RA3-6B2) antibody.
Size	For 2×10^9 total cells, up to 200 separations.
Product format	CD45R (B220) MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First, the CD45R$^+$ cells are magnetically labeled with CD45R (B220) MicroBeads. Then, the cell suspension is loaded onto a column which is placed in the magnetic field of a MACS® Separator. The magnetically labeled CD45R$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD45R$^+$ cells. After removal of the column from the magnetic field, the magnetically retained CD45R$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD45R (B220) MicroBeads were developed for positive selection or depletion of B cells from lymphoid tissue, peripheral blood or bone marrow. The CD45R antigen is expressed on B lymphocytes throughout their development from early pro-B stages on and is down-regulated upon terminal differentiation to plasma cells. CD45R is commonly used as a pan B cell marker.[1,2] It is also expressed on a small subset of dendritic cells (plasmacytoid DC).

Examples of applications

- Isolation of mouse B cells from lymph node and peritoneal cavities for studying cytokine expression,[3] antigen-presenting activity[4] or from spleen to study light chain gene rearrangement[5] and B cell proliferation.[6]
- Isolation of untouched macrophages[7,8] in combination with CD90 (Thy1.2) or CD4 (L3T4) and CD8 (Ly-2).
- Isolation of mouse B cells for adoptive transfer experiments to study the mouse model of leishmaniasis.[9]

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 in autoMACS™ Rinsing Solution (# 130-091-222). 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, 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: CD45R$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). CD45R (B220) MicroBeads can be used for depletion of CD45R$^+$ cells on LD, CS or D Columns. Cells which strongly express the CD45R 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 VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated CD45R antibodies, e.g. CD45R-FITC (# 130-091-829), CD45R-PE (# 130-091-828) or CD45R-APC (# 130-091-843).
- (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 (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ 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. Pipette off supernatant completely.
3. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD45R (B220) MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.
   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add a fluorochrome-conjugated CD45R antibody according to manufacturer's recommendation, and incubate for 5 minutes at 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. Pipette off 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 up to 1.25×10^8 cells in 500 µL of buffer.
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 $CD45R^+$ cells (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 (see "Column data sheets").
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 which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once 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 appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL.
   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed 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 (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 which 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 (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 onto the column.
4. Collect unlabeled cells which 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".

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.

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2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Possel"

   Depletion: "Depletes"

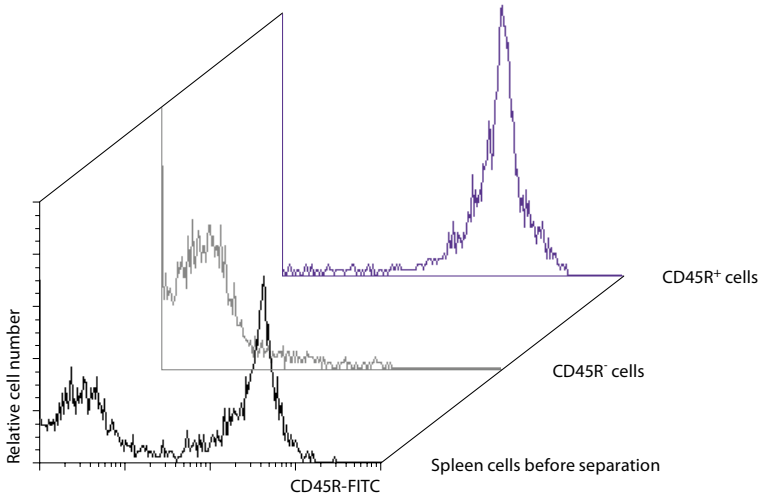
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".

3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CD45R$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD45R$^-$ cell fraction.

3. Example of a separation using CD45R (B220) MicroBeads

CD45R$^+$ cells were isolated from a mouse spleen cell suspension using CD45R MicroBeads, a MiniMACS™ Separator and an MS Column. The cells were fluorescently stained with CD45R-FITC (# 130-091-829). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Coffmann, B. (1982) Surface antigen expression and immunoglobulin rearrangement during mouse pre-B cell developement. Immunological Rev. 69: 5–23.
2. Ballas, Z. K. and Rasmussen, W. (1993) Lymphokine-activated killer cells VII. IL-4 induces an NK1.1$^+$CD8α$^+$β$^-$TCR-αβ B220$^+$ lymphokine-activated killer subset. J. Immunol. 150: 17–30
3. Hoerauf, A. *et al.* (1994) The Xid Defect Determines an Improved Clinical Course of Murine Leishmaniasis in Susceptible Mice. Int. Immunol. 6: 1117–1124. [61]
4. Guéry, J. *et al.* (1996) Dendritic Cells but not B Cells Present Antigenic Complexes to Class II-Restricted T Cells after Administration of Protein in Adjuvant. J. Exp. Med. 183: 751–757. [185]
5. Takeda, S. *et al.* (1993) Deletion of Immunoglobulin Kappa Chain Intron Enhancer Abolishes Kappa Chain Gene Rearrangement in cis but not γ Chain Gene Rearrangement in trans. EMBO J. 12: 2329–2336. [118]
6. Stüber, E. *et al.* (1995) Cross-Linking of OX40 Ligand, a member of the TNF/ NGF Cytokine Family, Induces proliferation and Differentiation in Murine Splenic B Cells. Immunity 2: 507–521. [174]
7. Vieth, M. *et al.* (1994) Interleukin-10 Inhibits Antimicrobial Activity Against Leishmania major in Murine Macrophage. Scand. J. Immunol. 40: 403–409. [126]
8. Neurath, M. F. *et al.* (1996) Local Administration of Antisense Phosphorothiate Oligonucleotides to the p65 Subunit of NK-κB Abrogates Established Experimental Colitis in Mice. Nature Medicine 2: 998–1004. [336]
9. Hoerauf, A. *et al.* (1996) Co-transfer of B Cells Converts Resistance into Suceptibility in T Cell-Reconstituted Leishmania major-Resistant C.B-17 SCID Mice by a Non-Cognate Mechanism. Internat. Immunol. 8: 1569–1575. [329]

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. MILTENYI BIOTEC GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. MILTENYI BIOTEC GmbH's liability is limited to either replacement of the products or refund of the purchase price. MILTENYI BIOTEC GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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