Isolation of rat B lymphocytes

Order No. 130-047-401

Index

1. Description

Components	2 mL Mouse Anti-Rat Kappa MicroBeads: MicroBeads conjugated to monoclonal anti-rat kappa antibodies (isotype: mouse IgG2a, κ; clone: MAR 18.5)
Size	For 10^9 total cells, up to 100 separations.
Product format	MACS® MicroBeads are supplied in a solution containing 0.1% gelatine 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

For MACS® separation, cells are magnetically labeled with Mouse Anti-Rat Kappa MicroBeads and separated on a column which is placed in the magnetic field of a MACS separator. The magnetically labeled Igκ$^+$ B cells are retained in the column while the unlabeled Igκ$^-$ B cells pass through. After removal of the column from the magnetic field, the magnetically retained Igκ$^+$ B cells can be eluted as the positively selected cell fraction. Igκ$^+$ cells can be enriched and also depleted from single cell suspensions by using MS, LS or XS Columns. However, most efficient depletion is achieved with LD, CS or D Columns. Positive selection and depletion can also be performed by using the autoMACS™ Separator.

1.2 Background and product applications

Mouse Anti-Rat Kappa MicroBeads can be used for the positive selection or depletion of rat B cells according to expression of immunoglobulin (Ig) kappa light chains. In B cell development, Ig light chains are first expressed, together with Ig heavy chain, on the surface of immature B cells (IgM$^+$IgD$^-$). They are present on naive (IgM$^+$IgD$^+$) and memory B cells and are down-regulated on activated B cells upon terminal differentiation to plasma cells. 95% of the rat B cells express kappa light chains.[1] Mouse Anti-Rat Kappa MicroBeads bind equally well to the Ig light chain allotypes Igκ-1a and Igκ-1b.[2] B cells can be isolated from blood, lymphoid and non-lymphoid tissue.

Examples of applications

B cells isolated with Mouse Anti-Rat Kappa MicroBeads from peripheral lymphoid organs or non-hematopoietic tissues are suitable for in vitro analyses on B cell development, differentiation and migration, for example. They are also used for in vivo studies after adoptive transfer of separated cells. Pure B cell populations also simplify molecular analyses e.g. on Ig-rearrangement.

1.3 Reagent and instrument requirements

- Buffer (degassed): PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% BSA (bovine serum albumin) 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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Fluorochrome-conjugated anti-rat kappa antibody
- (Optional) PI (propidium iodide) or 7-AAD for the exclusion of dead cells.
- (Optional) Pre-Separation Filter (# 130-041-407)
- MACS Columns and MACS Separators:
  Igκ$^+$ B cells can be enriched by using MS, LS or XS Columns (positive selection). Mouse Anti-Rat Kappa MicroBeads can be used for depletion of Igκ$^+$ B cells on LD, CS or D Columns. Cells which strongly express the Igκ 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	$2x10^8$	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	$2x10^9$	MidiMACS, VarioMACS, SuperMACS
XS	10^9	$2x10^{10}$	SuperMACS
Depletion			
LD	10^8	$5x10^8$	MidiMACS, VarioMACS, SuperMACS
CS	$2x10^8$		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	$2x10^8$	$4x10^9$	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

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2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Remove platelets after density gradient separation. Resuspend cell pellet, fill up with buffer and mix. Centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with tissue or body fluids, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

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

2.2 Magnetic labeling

▲ Work fast, keep the 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 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 Filter # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Mouse Anti-Rat Kappa MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.

   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add fluorochrome conjugated anti-rat kappa 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 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 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 Igκ$^+$ B cells (see table 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: 3×3mL.
   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 second, 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 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 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 magnetically labeled cells in autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Possel"

   Depletion: "Depletes"

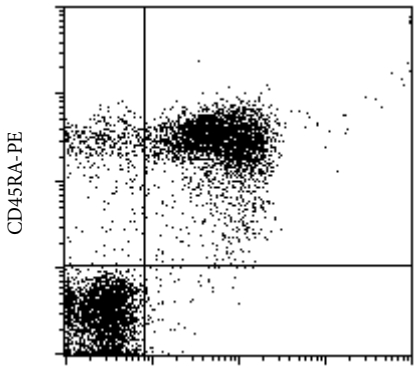
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using program "Possel", collect positive fraction. This is the purified Igκ$^+$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

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

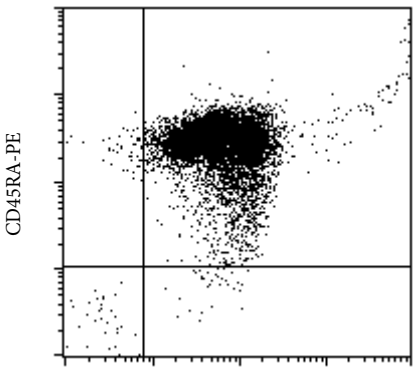
3. Example of a separation using Mouse Anti-Rat Kappa MicroBeads

Separation of B cells from rat spleen using Mouse Anti-Rat Kappa MicroBeads, a MiniMACS with an MS Column. The cells are fluorescently stained with mouse anti-rat kappa-FITC and CD45RA-PE.

Spleen cells before separation



Positive fraction



4. References

1. Bazin, H; Cormont, F; De Clercq, L. (1984) Rat monoclonal antibodies. II. A rapid and efficient method of purification from ascitic fluidor serum. J. Immunol. Methods 71: 9-16.
2. Lanier, LL; Gutman, GA; Lewis, DE; Grisworld, ST; Warner, NL (1982) Monoclonal antibodies against rat immunoglobulin kappa chains. Hybridoma 1: 125-31.

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

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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