Miltenyi Biotec

Anti-Red Blood Cell MicroBeads
rat

Order no. 130-109-681

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1. Description

This product is for research use only.

Components	1 mL Anti-Red Blood Cell MicroBeads, rat: MicroBeads conjugated to monoclonal anti-rat red blood cell antibodies (isotype: recombinant human IgG1).
Capacity	For 5×10^9 total cells, up to 50 separations.
Product format	Anti-Red Blood Cell MicroBeads are supplied in buffer containing stabilizer.
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 red blood cells are magnetically labeled with Anti-Red Blood Cell 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 red blood cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of red blood cells.

1.2 Background information

Anti-Red Blood Cell MicroBeads, rat have been developed for the separation of cells expressing a 52 kDa glycophorin A–associated protein, also known as Ly-76, as an alternative method to osmotic lysis. It is expressed on rat red blood cells and erythroid progenitors, but not on granulocytes, monocytes, or lymphocytes.

1.3 Applications

- Depletion of rat red blood cells , e.g., from rat lung, heart, fat, muscle, or spleen.

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. Always use freshly prepared buffer.

  ▲ **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 rat serum albumin, rat serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Red blood cells can be depleted with the use of LD Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II

  ▲ **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 antibodies for flow cytometric analysis, e.g., Anti-Erythroid Cells-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 (70 µm) (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.com/protocols.

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▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^8 total cells.
   ▲ **Note:** Always use freshly prepared buffer.
4. Add 20 µL of Anti-Red Blood Cell MicroBeads per 10^8 total cells.
5. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of Anti-Erythroid Cells-PE, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Adjust volume to 500 µL using buffer for up to 10^8 total cells. Do not centrifuge.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

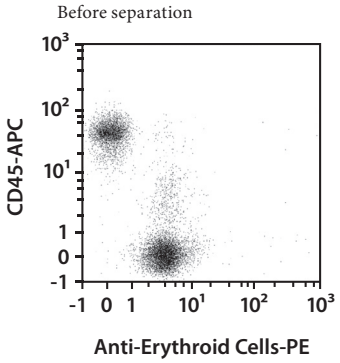
▲ Always wait until the column reservoir is empty before proceeding to the next step.

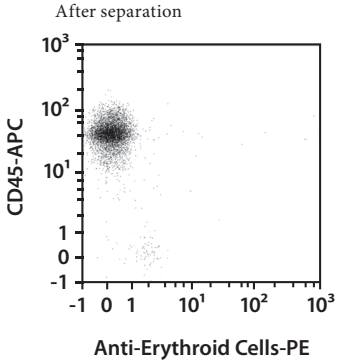
Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the 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 flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty

3. Example of a separation using Anti-Red Blood Cell MicroBeads

Red blood cells were depleted from a rat spleen cell suspension using Anti-Red Blood Cell MicroBeads, an LD Column, and a MidiMACS™ Separator. Cells were fluorescently stained with Anti-Erythroid Cells-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





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.

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