Miltenyi Biotec

CD8 MicroBead Kit
non-human primate

Order No. 130-091-112

Index

1. Description

Components	**1 mL CD8-PE, non-human primate:** monoclonal anti-human CD8 antibody conjugated to R-phycoerythrin (PE) (isotype: mouse IgG2a; clone: BW135/80) **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	CD8-PE is supplied in a solution containing 0.1% gelatine and 0.05% sodium azide. MACS® MicroBeads are supplied as a suspension 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.

This product is applicable for the separation of cells from rhesus monkey (*Macaca mulatta*). The CD8 antibody has been tested to cross-react with cynomolgus monkey (*Macaca fascicularis*). Cross-reactivity with other non-human primates has not been tested.

1.1 Principle of MACS® separation

First the $CD8^+$ cells are stained with CD8-PE. Subsequently, the cells are magnetically labeled with Anti-PE 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 $CD8^+$ cells are retained in the column. The unlabeled cells run through, this cell fraction is depleted of $CD8^+$ cells. After removal of the column from the magnetic field, the magnetically retained $CD8^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

The CD8 MicroBead Kit is developed for the separation of non-human primate cells based on the expression of the CD8 antigen. CD8 is expressed on rhesus monkey cytotoxic T cells and NK cells.[1] For the isolation of highly pure cytotoxic T cells, NK cells can be depleted by using CD16 MicroBeads, non-human primate (# 130-091-145).

Examples of applications

- Positive selection or depletion of cells expressing CD8 antigen.
- Isolation or depletion of cytotoxic T cells from rhesus monkey peripheral blood mononuclear cells (PBMCs) or single-cell suspensions from lymphoid tissue.

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 with 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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: $CD8^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). CD8 MicroBeads can be used for depletion of $CD8^+$ cells on LD, CS or D Columns. Cells which strongly express the CD8 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 or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) FcR Blocking Reagent (# 130-059-901) to avoid Fc receptor-mediated cell staining.
- (Optional) PI (propidium iodide) or 7-AAD for the flow- cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-000-830.05

2. Protocol

2.1 Sample preparation

When working with rhesus monkey anticoagulated peripheral blood or buffy coat, PBMCs can be isolated by density gradient centrifugation, e.g. Ficoll-Paque™, as with human samples (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and 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, prepare a single-cell suspension by using a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or using 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 a 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 Filters # 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 10^7 total cells in 100 µL buffer and add 10 µL CD8-PE.
   ▲ **Note:** FcR Blocking Reagent can be added to inhibit non-specific or Fc-receptor mediated binding of antibody to non-target cells: resuspend 10^7 total cells in 80 µL of buffer, add 20 µL FcR Blocking Reagent and 10 µL CD8-PE.
4. Mix well and incubate for 10 minutes in the dark 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.
5. Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes.
6. (Optional) Repeat washing step.
7. Pipette off supernatant completely and resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
9. 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.
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes.
11. Pipette off supernatant completely.
12. 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 up to 1.25×10^8 cells in 500 µL of buffer.
13. 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 $CD8^+$ 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 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 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 autoMACS™ Separator

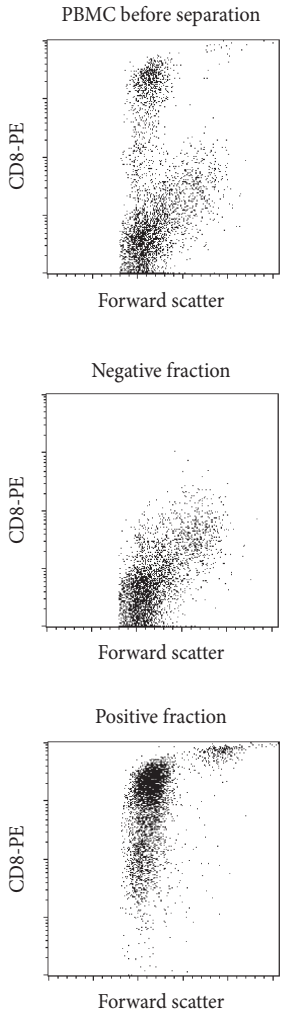
▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing magnetically labeled cells in autoMACS Separator. Choose a separation program according to the recommendations in the autoMACS User Manual.

   ▲ **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. Example of a separation using CD8 MicroBead Kit

Separation of rhesus monkey PBMCs using MACS CD8 MicroBead Kit and a MiniMACS™ Separator with an MS Column. The cells are fluorescently stained with CD8-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. Reference

1. Carter, DL; Shieh, TM; Blosser, RL; Chadwick, KR; Margolick, JB; Hildreth, JEK; Clements, JE; Zink, MC (1999) CD56 Identifies Monocytes and Not Natural Killer Cells in Rhesus Macaques. Cytometry 37: 41–50.

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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