CD137 MicroBead Kit
human

Order no. 130-093-476

Contents

1. Description

Components	**1 mL CD137-PE, human:** Monoclonal anti-human CD137 antibody conjugated to R-phycoerythrin (PE) (clone: 4B4-1, isotype: mouse IgG1). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibody
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
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 CD137$^+$ cells are indirectly magnetically labeled with CD137-PE antibody and 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 CD137$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD137$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD137$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The activation-induced antigen CD137 (4-1BB) is a 30 kDa glycoprotein of the tumor necrosis factor (TNF) receptor superfamily. It is mainly expressed on activated CD4$^+$ and CD8$^+$ T cells, activated B cells, and natural killer cells, but can also be found on resting monocytes and dendritic cells.
As a costimulatory molecule, CD137 is involved in the activation and survival of CD4, CD8, and NK cells. Its engagement enhances expansion of T cells and activates them to secrete cytokines.
CD137 has been described to be a suitable marker for antigen-specific activation of human CD8$^+$ T cells, as CD137 is not expressed on resting CD8$^+$ T cells and its expression is reliably induced after 24 hours of stimulation.[1,2]

1.2 Applications

- Enrichment of CD137$^+$ T cells for phenotypical and functional characterization.
- Enrichment of activated antigen-specific T cells after antigen-specific stimulation.

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.

  ▲ **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 bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD137$^+$ cells can be enriched by using MS or LS Columns. Positive selection can also be performed by using the autoMACS or the autoMACS Pro 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
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD8-FITC (# 130-080-601) or CD8-APC (# 130-091-076). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

140-002-200.03

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- (Optional) Stimulation reagents: CytoStim (# 130-092-172, # 130-092-173) as control reagent for T cell stimulation and, e.g., PepTivator – CMV pp65 (# 130-093-435, # 130-093-438) or the CMV pp65 – Recombinant Protein (# 130-091-823, # 130-091-824) for antigen-specific T cell stimulation. For more information about other antigens see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) or 7-AAD 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 (# 130-041-407) 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™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ 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).

2.1.1 Protocol for *in vitro* stimulation for induction of CD137 expression

▲ Always include a negative control in the experiment. The sample should be treated exactly the same way as the stimulated sample, except for the addition of the stimulus.

▲ A positive control may also be included in the experiment, such as a sample stimulated with CytoStim (# 130-092-172).

▲ Do not use media containing any non-human proteins, such as BSA or FCS, because of non-specific stimulation.

1. Wash cells by adding cell culture medium, centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cells at a density of 10^7 cells/mL in culture medium containing 5% human serum. Plate cells in dishes at a density of 5×10^6 cells/cm^2 (see Appendix).
3. Add an antigen or control reagent in the appropriate concentration, for example, PepTivator – CMV pp65 (# 130-093-435, # 130-093-438).
4. Incubate cells overnight with antigen and an appropriate control, e.g., CytoStim, at 37 °C and 5% CO_2.

   ▲ **Note:** Stimulation for 4–6 hours with CytoStim is sufficient to induce CD137 expression. For antigen-specific stimulation an overnight incubation is recommended.
5. Collect cells carefully by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells. If necessary, rinse the dish again.

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. Wet filter with buffer before use.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD137-PE per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 5 µL of CD8-FITC (# 130-080-601), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 0.5–1 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
9. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
10. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
11. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
13. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD137$^+$ cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of CD137$^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with the autoMACS™ Separator or the autoMACS™ Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Separator

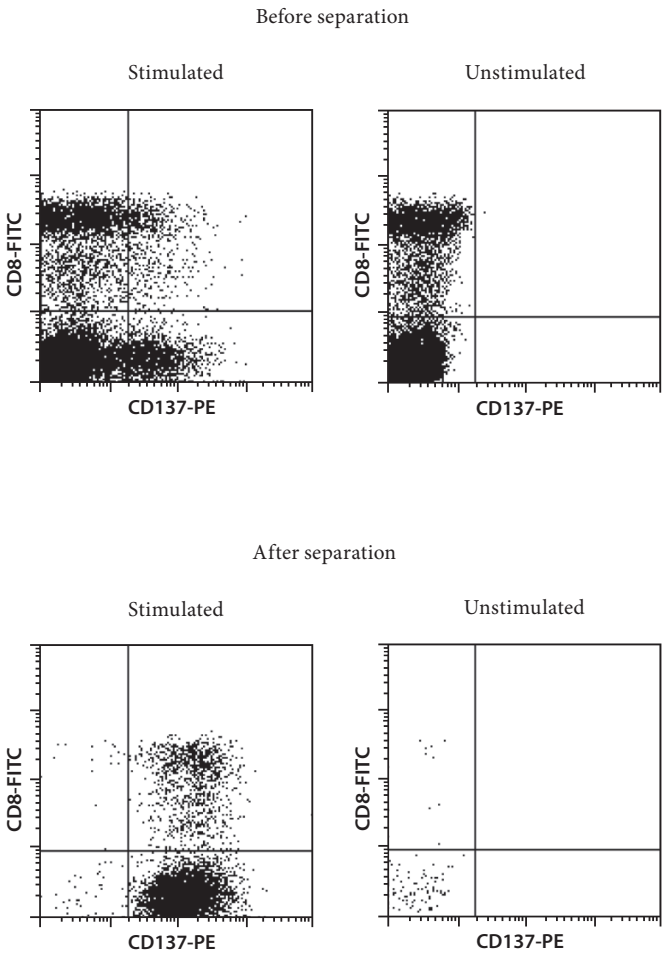
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose the following program:
   Positive selection: "Posselds"
   Collect positive fraction from outlet port pos2.

Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:
   Positive selection: "Posselds"
   Collect positive fraction in row C of the tube rack.

3. Example of a separation using the CD137 MicroBead Kit

CD137$^+$ cells were isolated from human PBMCs of a CMV$^+$ donor using the CD137 MicroBead Kit, two MS Columns, and a MiniMACS™ Separator. PBMCs were incubated overnight with and without a CMV pp65 peptide pool (PepTivator – CMV pp65, # 130-093-435). Subsequently, cells were labeled with CD137-PE and Anti-PE MicroBeads and magnetically separated. Cell fractions are additionally fluorescently stained with CD8-FITC (# 130-080-601). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Wehler, T. C. *et al.* (2007) Targeting the activation-induced antigen CD137 can selectively deplete alloreactive T cells from antileukemic and antitumor donor T-cell lines. Blood 109(1): 365–373.
2. Wolfl, M. *et al.* (2007) Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of $CD8^+$ T cells responding to antigen without requiring knowledge of epitope specificities. Blood 110(1): 201–210:

All protocols and data sheets are available at www.miltenyibiotec.com.

5. Appendix: Flask and dish sizes for *in vitro* stimulation of PBMCs

For *in vitro* stimulation of PBMCs (see 2.2) the cells should be resuspended in culture medium, containing 5% of human serum, at a dilution of 10^7 cells/mL. The cells should be plated at a density of 5×10^6 cells/cm². Both the dilution and the cell density are important to assure optimum stimulation.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
0.15×10^7	0.15 mL	96 well	0.64 cm
0.50×10^7	0.50 mL	48 well	1.13 cm
1.00×10^7	1.00 mL	24 well	1.60 cm
2.00×10^7	2.00 mL	12 well	2.26 cm
5.00×10^7	5.00 mL	6 well	3.50 cm
Total cell number	**Medium volume to add**	**Culture dish**	**Dish diameter**
4.5×10^7	4.5 mL	small	3.5 cm
10.0×10^7	10.0 mL	medium	6 cm
25.0×10^7	25.0 mL	large	10 cm
50.0×10^7	50.0 mL	extra large	15 cm
Total cell number	**Medium volume to add**	**Culture flask**	**Growth area**
12×10^7	12 mL	50 mL	25 cm²
40×10^7	40 mL	250 mL	75 cm²
80×10^7	80 mL	720 mL	162 cm²
120×10^7	120 mL	900 mL	225 cm²

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

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140-002-200.03