



Miltenyi Biotec

Multi-Parameter Magnetic Cell Sorting

Components

MACS colloidal super-paramagnetic MultiSort MicroBeads conjugated to monoclonal mouse anti-human CD19 antibody. Isotype: mouse IgG1. MultiSort Release Reagent for enzymatic release of MultiSort MicroBeads bound to the cell surface. MultiSort Stop Reagent to inhibit the release reaction for further separations.

Application

CD19 MultiSort MicroBeads were developed for isolation of CD19⁺ B cell subpopulations. CD19 is expressed on precursor B cells and B cells.

- ▲ Positive selection of cells in suspension expressing the CD19 antigen and subsequent release of the magnetic label in order to magnetically isolate subpopulations of CD19⁺ cells according to expression of additional surface markers.

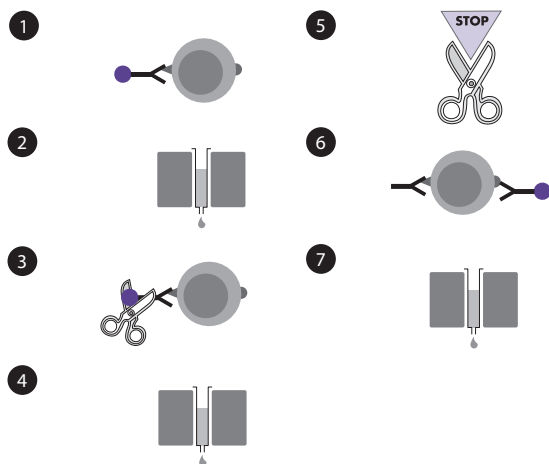
Storage of MACS MultiSort Reagents

Store protected from light at 4°C. Do not freeze.

Instrument and Reagent Requirement

Magnetic cell separators MiniMACS, MidiMACS, VarioMACS or SuperMACS (plus RS⁺ or VS⁺ column adaptor). Positive selection columns type MS⁺/RS⁺ or LS⁺/VS⁺. Buffer: phosphate buffered saline supplemented with 0.5 % bovine serum albumin and 2 mM EDTA, pH 7.2 (see "Important Notes"). (Optional) Fluorochrome conjugated CD19 antibody.

Step-by-step CD19 MultiSort Separation Procedure



CD19 MultiSort Kit

2 ml MultiSort CD19 MicroBeads

1 ml MultiSort Release Reagent

2 ml MultiSort Stop Reagent

For 1×10⁹ total cells

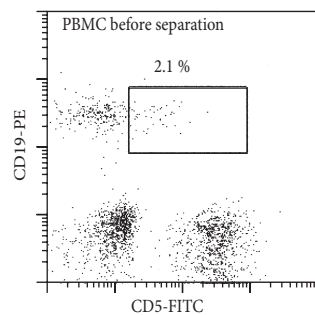
Order No. 130-055-301

Description of MACS CD19 MultiSort Kit

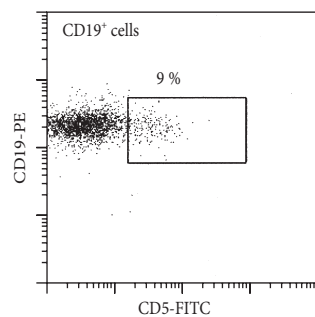
With the CD19 MultiSort Kit B cell subpopulations can be isolated according to multiple surface markers. Cells are magnetically labeled using MACS CD19 MultiSort MicroBeads (1). Labeled cells are enriched on positive selection columns (2). The MultiSort Release Reagent releases the MultiSort MicroBeads from the cells of the positive fraction (3). The primary antibody remains on the cells. Release of the MultiSort MicroBeads allows a second magnetic labeling and separation of the cells for another surface marker of interest (4-7) using direct MACS MicroBeads, indirect MACS Streptavidin MicroBeads or indirect MACS Anti-FITC MicroBeads.

Example for a Separation Using MACS CD19 MultiSort Kit

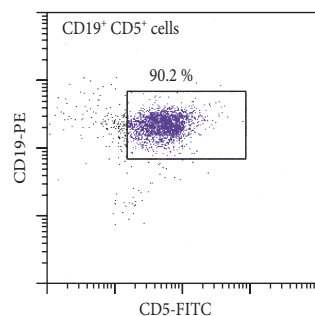
Isolation of human peripheral blood CD19⁺CD5⁺ cells using MACS CD19 MultiSort Kit and MS⁺ columns for the first positive selection. After release of the magnetic label, the positive selection for the second marker is performed with FITC-conjugated CD5 antibody, MACS Anti-FITC MicroBeads and MS⁺ columns.



PBMC are labeled with MultiSort CD19 MicroBeads (1), CD19-phycoerythrin (PE) and CD5-FITC.



CD19⁺ cells are positively selected using a MS⁺ column and MiniMACS (2). The MicroBeads are released (3) and the non-released cells removed by separation over a MS⁺ column in MiniMACS (4). The release reaction is stopped (5). The released fraction is labeled with Anti-FITC MicroBeads (6).



CD19⁺CD5⁺ double positive cells are positively selected from preselected CD19⁺ cells using CD5-FITC, Anti-FITC MicroBeads and MS⁺ columns in MiniMACS (7).

140-000-002-04

Protocol for Magnetic Labeling of Cells in Suspension

- Isolate single cell suspension by standard preparation method. To remove clumps, pass cells through 30 µm nylon mesh or pre-column separation filter (Order No. 130-041-407). Wet filters with buffer before use. Dead cells can be removed by density gradient centrifugation (e.g. Ficoll-Paque™).
- Wash cells carefully, discard supernatant and resuspend cell pellet in 80 µl of buffer per 10⁷ total cells. For fewer cells, use same volume.
- Label cells by adding 20 µl CD19 MultiSort MicroBeads per 10⁷ total cells, mix well and incubate for 15 minutes in refrigerator at 6°–12°C.
- When an indirect system is used for second parameter separation, incubate simultaneously with primary antibody (biotinylated or FITC-conjugated) for second surface antigen at a titer recommended by the manufacturer for the last 10 minutes.
- (Optional) Add fluorochrome conjugated CD19 antibody at the titer recommended by manufacturer and incubate for further 5–10 minutes.
- Wash cells carefully and resuspend in appropriate amount of buffer (MS⁺/RS⁺ column: 500–1000 µl; LS⁺/VS⁺ column: 1–10 ml, max. 2×10⁸ cells per ml).
- Proceed to magnetic separation.

Magnetic Separation with MACS (Protocol for 10⁴–10⁸ Positive Cells)

- Choose a positive selection column type MS⁺/RS⁺ (for up to 10⁷ positive cells), or LS⁺/VS⁺ (for up to 10⁸ positive cells) and place the column in separator.
- Prepare the column by washing with appropriate amount of buffer (MS⁺/RS⁺: 500 µl, LS⁺/VS⁺: 3 ml; for details, see "Column Data Sheets").
- Apply cell suspension in appropriate amount of buffer to the column (MS⁺/RS⁺: 500–1000 µl, LS⁺/VS⁺: 1–10 ml, max. 2×10⁸ cells per ml). Let the negative cells pass through. Rinse with appropriate amount of buffer (MS⁺/RS⁺: 3×500 µl, LS⁺/VS⁺: 3×3 ml).
- Remove column from separator, place column on a suitable collection tube, pipette appropriate amount of buffer (MS⁺/RS⁺: 1 ml; LS⁺/VS⁺: 5 ml) to the column and flush out positive fraction using the plunger supplied with the column.
- (Optional) To achieve a higher purity, apply positive fraction to a new, freshly prepared column. Let the negative cells pass through. Rinse with appropriate amount of buffer (MS⁺/RS⁺: 3×500 µl, LS⁺/VS⁺: 3×3 ml). Elute positive fraction as described above.
- Remove a sample to analyse the separation by flow cytometry. With remaining positive fraction subsequently proceed to "Removal of MACS MultiSort MicroBeads".

Removal of MACS MultiSort MicroBeads Using MACS MultiSort Release Reagent

- Incubate magnetically selected cells with 20 µl MACS MultiSort Release Reagent per ml cell suspension for 10 minutes in refrigerator at 6°–12°C.
- (Optional) Separate cells over a new column of the same type as will be used for the second parameter separation to remove any remaining magnetically labeled cells.
- Wash cells from the released fraction, remove supernatant completely and resuspend cell pellet in buffer in a final volume of 50 µl per 10⁷ cells, for less cells resuspend in 50 µl of buffer.
- For every 10⁷ cells add 30 µl of MACS MultiSort Stop Reagent and mix well, for less cells use 30 µl of MACS MultiSort Stop Reagent.
- For every 10⁷ cells add the recommended amount of direct MACS MicroBeads (see "MicroBead Data Sheets"), 10 µl MACS Streptavidin MicroBeads or 10 µl MACS Anti-FITC MicroBeads to magnetically label the cells for the second marker. Use buffer to adjust to 100 µl labeling volume and incubate for 15 minutes in refrigerator at 6°–12°C. For less than 10⁷ cells adjust to 100 µl labeling volume.
- Cells can now be separated according to the second marker by using positive selection columns.

Important Notes

- ▲ EDTA can be replaced by other supplements such as acid citrate dextrose (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as gelatin, HSA or FCS. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended.
- ▲ Avoid capping of antibodies on the cell surface during labeling. Work fast, keep cells cold, use cold solutions only.
Attention: Working on ice requires increased incubation times for MACS MicroBeads. Incubate in refrigerator at 6°–12°C.
- ▲ Higher temperatures and longer incubation times for magnetic labeling may lead to unspecific cell labeling.
- ▲ MACS MicroBeads may bind unspecifically to dead cells. Therefore, dead cells should be removed before labeling, e.g. by Ficoll-Paque™ density gradient centrifugation.
- ▲ Large numbers of cells in the starting sample require a larger buffer volume when applying cells to the separation column. Use a maximum cell concentration of 10⁸ cells per 500 µl of buffer.
- ▲ To increase purity of the positive fractions, cells can be separated a second time over a new positive selection column.
- ▲ We recommend using either direct MACS MicroBeads, indirect Anti-FITC or Streptavidin MicroBeads for second parameter sorting. Anti-Immunoglobulin MicroBeads can also be used, but take into account that the CD19 antibody is of mouse IgG1 isotype. Goat-Anti-Mouse IgG or Rat-Anti-Mouse IgG1 MicroBeads cannot be used.

- ▲ If cells to be separated according to the second parameter are rare (< 1 %) in the positively selected fraction of the first sort, remove remaining magnetically labeled cells after release of the MultiSort MicroBeads by MACSing over a separation column. To increase purity of target cells according to the second parameter, cells can be separated a second time over a new column.

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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