

Index

1. Description
 - 1.1 Principle of MACS® Separation
 - 1.2 Background and product applications
 - 1.3 Reagent and instrument requirements
2. Protocol
 - 2.1 Sample preparation
 - 2.2 Magnetic labeling
 - 2.3 Magnetic separation
3. Example of a separation using Anti-HLA-DR MicroBeads
4. Appendix: Lysis of erythrocytes

1. Description

Components	2 mL Anti-HLA-DR MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti HLA-DR antibodies (isotype: mouse IgG1).
Size	For 10 ⁹ total cells, up to 100 separations.
Product format	Anti-HLA-DR MicroBeads are supplied as a suspension containing 0.1% 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 MACS® Separation

First, the HLA-DR⁺ cells are magnetically labeled with Anti-HLA-DR 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 HLA-DR⁺ cells are retained on the column. The unlabeled cells run through; this cell fraction is thus depleted of HLA-DR⁺ cells. After removing the column from the magnetic field, the magnetically retained HLA-DR⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Anti-HLA-DR MicroBeads are developed for the separation of cells based on the expression of the HLA-DR antigen. HLA-DR is expressed on dendritic cells, B cells, monocytes, macrophages, activated T cells, activated NK cells, hematopoietic progenitor cells and some epithelial cells.

Example applications

- Positive selection or depletion of antigen presenting cells, e.g., B cells, monocytes and dendritic cells, from human PBMCs (peripheral blood mononuclear cells) or lymphoid tissue.
- Isolation of primitive CD34⁺ HLA-DR⁻ hematopoietic progenitor cells in combination with CD34 MultiSort Kit (# 130-056-701) from human peripheral blood, cord blood or bone marrow.

- Positive selection of HLA-DR expressing cells from human PBMCs or buffy coat for serological typing of HLA-DR class II molecules.

1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: HLA-DR⁺ cells can be enriched by using MS, LS or XS Columns (positive selection) or depleted by using LD, CS or D Columns. Cells which strongly express the HLA-DR antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Separator.

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

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

- (Optional) Fluorochrome-conjugated Anti-HLA-DR antibody for flow-cytometric analysis.
- (Optional) Propidium iodide (PI) or 7-AAD for flow-cytometric exclusion 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, e.g., using Ficoll-Paque™. For details see section General Protocols in the user manuals or visit 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, prepare a single-cell suspension by a standard preparation method. For details see section 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 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 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ 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.

Magnetic labeling of human PBMCs or cells from lymphoid tissue

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10⁷ total cells.
4. Add 20 µL of Anti-HLA-DR MicroBeads per 10⁷ total cells.
5. Mix well and refrigerate for 15 minutes at 4–8 °C.

▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add staining antibodies according to manufacturer's recommendation and refrigerate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10⁸ cells in 500 µL of buffer.

▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10⁸ cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

Magnetic labeling of cells from buffy coat

1. Centrifuge 2–3 mL anticoagulated peripheral blood at 400×g for 35 minutes. Carefully collect leukocyte enriched interphase (buffy coat) in 300 µL volume.

▲ **Note:** For lysis of erythrocytes see 4. Appendix.
2. Add 60 µL of Anti-HLA-DR MicroBeads per 300 µL of buffy coat.
3. Mix well and refrigerate for 20 minutes at 4–8 °C.

▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. (Optional) Add staining antibodies according to manufacturer's recommendation and refrigerate for 5 minutes at 4–8 °C.
5. Wash cells by adding 3–6 mL of buffer and centrifuge at 200×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10⁸ cells in 500 µL of buffer.

▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10⁸ cells in 500 µL of buffer.
7. 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 HLA-DR⁺ cells. For details see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:

MS: 500 µL	LS: 3 mL
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3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. 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
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 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 an 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
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▲ **Note:** To increase the purity of the magnetically labeled fraction, pass the cells 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. For details 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 that 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. For details 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. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the 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.

1. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
2. Resuspend all pellet in 40 μ L of buffer per 10^7 total cells.
3. Add 60 μ L of MultiSort Stop Reagent per 10^7 total cells and mix well.
4. Add 100 μ L Anti-HLA-DR MicroBeads per 10^7 total cells.
5. Mix well and refrigerate for 15 minutes at 4–8 °C.

Magnetic separation with the 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 the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose one of the following separation programs:

Positive selection: "Possel"

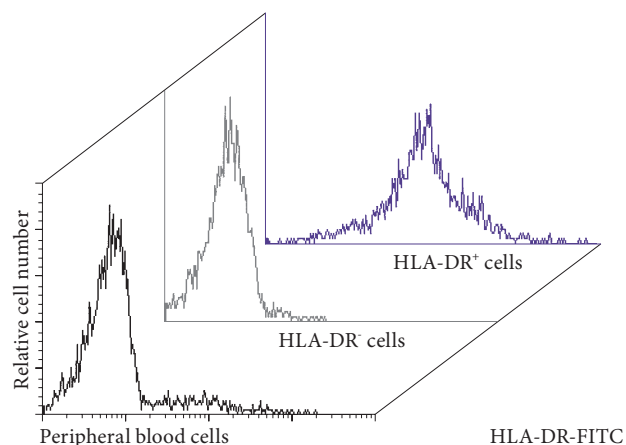
Depletion: "Depletes"

▲ **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, section autoMACS Cell Separation Programs.

3. When using the program "Possel", collect positive fraction from outlet port "pos1". This is the purified HLA-DR⁺ cell fraction.
When using the program "Depletes", collect unlabeled fraction from outlet port "neg1". This is the HLA-DR⁻ cell fraction.

3. Example of a separation using Anti-HLA-DR MicroBeads

Separation of human leukocyte enriched buffy coat using Anti-HLA-DR MicroBeads and a MiniMACS™ Separator with an MS Column. Cells are fluorescently stained with Anti-HLA-DR-FITC.



4. Appendix: Lysis of erythrocytes

If buffy coat was used for the isolation of HLA-DR⁺ cells remaining erythrocytes can be lysed.

1. Incubate the buffy coat with an excess volume of isotonic ammonium chloride buffer (155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA) for 5–7 minutes at room temperature.
2. Centrifuge cells at $300\times g$ for 15 minutes at 20 °C.
3. Wash cells twice with buffer.
4. Resuspend cell pellet in buffer.

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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