Miltenyi Biotec

CD209 (DC-SIGN) MicroBead Kit
human

Order no. 130-092-868

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1. Description

Components	**2 mL CD209 (DC-SIGN) MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD209 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent:** human IgG.
Size	For 2×10^9 total cells, up to 20 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 MACS® Separation

First, the CD209 (DC-SIGN)$^+$ cells are magnetically labeled with CD209 (DC-SIGN) 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 CD209 (DC-SIGN)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD209 (DC-SIGN)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD209 (DC-SIGN)$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD209 (Dendritic Cell Specific ICAM-3 Grabbing Non-integrin, DC-SIGN) is a type II C-type lectin receptor (CLR). It is expressed on DCs in dermal and mucosal tissue, in lymphoid tissue such as tonsil, lymph node, and spleen, and on monocyte-derived DCs (Mo-DCs). It is not expressed on DC subsets in peripheral blood, except for a subpopulation of CD14$^+$ cells with a DC-like phenotype.[1]

DC-SIGN acts as an adhesion receptor to facilitate interactions between DCs and T cells or DCs and endothelial cells. In addition, it also serves as an antigen receptor to mediate internalization of ligands for antigen presentation, binding viruses such as HIV, HCMV, or Ebola as well as parasites, bacteria, or yeast. HIV-1 uses this feature of DC-SIGN for efficient in-*trans* infection of CD4$^+$ T cells.[1,2]

Example application

- Positive selection or depletion of cells expressing the human CD209 (DC-SIGN) antigen.

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD209 (DC-SIGN)$^+$ cells can be enriched by using MS, LS, or XS Columns (positive selection) or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD209 (DC-SIGN) 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™ Separators. 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 CD209 (DC-SIGN) antibody for flow cytometric analysis, e.g. CD209 (DC-SIGN)-FITC (# 130-092-873), CD209 (DC-SIGN)-PE (# 130-092-869), or CD209 (DC-SIGN)-APC (# 130-092-871).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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- (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 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 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 cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
4. Add 100 µL of CD209 (DC-SIGN) MicroBeads per 10^8 total cells.
5. Mix well and refrigerate for 15 minutes (4–8 °C).

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. (Optional) Add staining antibodies, e.g. 50 µL of CD209 (DC-SIGN)-FITC (# 130-092-873), and refrigerate for 5 minutes in the dark (4–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 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 1.25×10^8 cells in 500 µL of buffer.
9. 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 CD209 (DC-SIGN)$^+$ 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
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
   Collect total effluent; this is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of eluting into a 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
7. To increase the purity of CD209$^+$ cells, the eluted fraction should be enriched over a second column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new MS or LS 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.

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: "Posseld2"

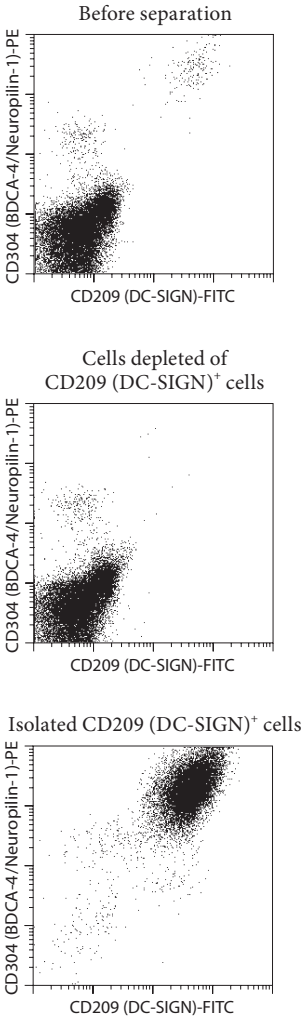
   Depletion: "Depletes"

   ▲ **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 "Posseld2", collect positive fraction from outlet port pos2. This is the purified CD209 (DC-SIGN)$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction from outlet port neg1. This is the CD209 (DC-SIGN)$^-$ cell fraction.

3. Example of a separation using CD209 (DC-SIGN) MicroBead Kit

Separation of CD209$^+$ cells from human peripheral blood mononuclear cells (PBMCs) spiked with 1% Mo-DCs using the CD209 MicroBead Kit, two MS Columns and a MiniMACS™ Separator. Mo-DCs were generated *in vitro* with GM-CSF and IL-4 for 7 days. Cells were stained with CD209 (DC-SIGN)-FITC and CD304 (BDCA-4/Neuropilin-1)-PE (# 130-090-533) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Koppel, E. A. *et al.* (2005) Distinct functions of DC-SIGN and its homologues L-SIGN (DC-SIGNR) and mSIGNR1 in pathogen recognition and immune regulation. Cell. Microbiol. 7: 157–165.
2. Geijtenbeek, T. B. *et al.* (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell. 2000 Mar 3; 100(5): 575–585.

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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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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