



CD209 (DC-SIGN) antibodies human

CD209 (DC-SIGN)-FITC	130-092-873
CD209 (DC-SIGN)-PE	130-092-869
CD209 (DC-SIGN)-APC	130-092-871
CD209 (DC-SIGN)-Biotin	130-092-875
CD209 (DC-SIGN) pure	130-092-877

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1. Description

Clone DCN47.5 (isotype: mouse IgG1).

Product format 1 mL CD209 (DC-SIGN) antibodies, human: monoclonal CD209 (DC-SIGN) antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests or up to 10⁹ total cells.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 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 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.

DC-SIGN acts as an adhesion receptor to facilitate interactions between DCs and T cells or DCs and endothelial cells. In addition, it 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}

Product applications

- Identification and enumeration of CD209 (DC-SIGN)⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human CD209 (DC-SIGN)⁺ cells can be isolated by using, e.g., the CD209 (DC-SIGN) MicroBead Kit, human (# 130-092-868).

1.2 Recommended antibody dilution

For antibody labeling of human cells.

CD209 (DC-SIGN) conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11
- CD209 (DC-SIGN) MicroBead-labeled cells	1:11	1:11	1:11	1:11
Immunohistochemistry^b				

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) The optimal antibody dilution should be determined.

1.3 Reagent 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).
▲ **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.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD209 (DC-SIGN)-Biotin.
- (Optional) CD14-FITC (# 130-080-701), CD14-PE (# 130-091-242), or CD14-APC (# 130-091-243).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of the CD209 (DC-SIGN) antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

140-001-9141-01

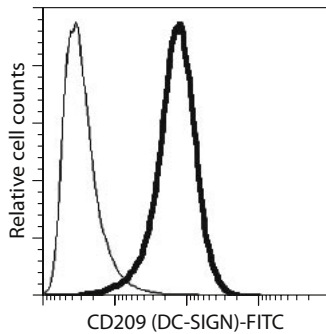


5. (Optional) If CD209 (DC-SIGN)-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC # 130-090-857, Anti-Biotin-PE # 130-090-756, or Anti-Biotin-APC # 130-090-856), and continue as described in steps 3 and 4.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

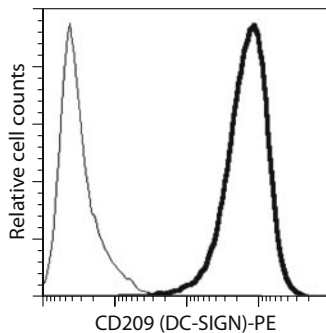
3. Examples of immunofluorescent staining with CD209 (DC-SIGN) antibodies

Human monocyte-derived dendritic cells (Mo-DCs), generated *in vitro* with GM-CSF and IL-4 for 7 days, were stained with CD209 (DC-SIGN) antibodies conjugated to FITC (a), PE (b), or APC (c) and analyzed by flow cytometry. Cells stained with CD209 (DC-SIGN)-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. Black line represents staining with CD209 (DC-SIGN) antibodies, grey line represents isotype control.

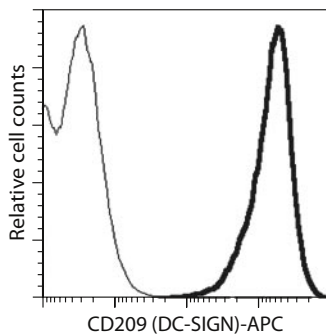
(a) Human Mo-DCs stained with CD209 (DC-SIGN)-FITC.



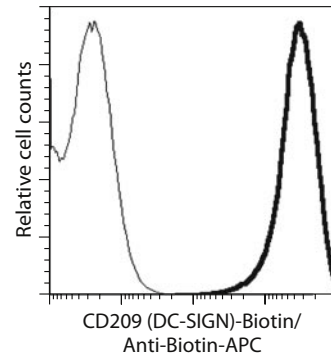
(b) Human Mo-DCs stained with CD209 (DC-SIGN)-PE.



(c) Human Mo-DCs stained with CD209 (DC-SIGN)-APC.



(d) Human Mo-DCs stained with CD209 (DC-SIGN)-Biotin and Anti-Biotin-APC.



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* 100: 575-585.

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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