

Product Information

Monoclonal Anti-DC-SIGN1

Clone 120507

produced in mouse, purified immunoglobulin

Catalog Number **D2191**

Product Description

Monoclonal Anti-DC-SIGN1, Clone 120507 (mouse IgG2B) is produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with a NIH/3T3 transfectant stably expressing human DC-SIGN1. The IgG fraction of the tissue culture supernatant is purified by Protein G affinity chromatography.

Monoclonal Anti-DC-SIGN1 recognizes human DC-SIGN1 on transfected NIH/3T3 cells and on monocyte derived dendritic cells. Applications include flow cytometry, adhesion inhibition, immunohistochemistry, and immunoblotting. The antibody does not react with parental mouse cells or irrelevant transfectants, such as human DC-SIGN2.

Dendritic cells (DC) play a primary role in the immune system as antigen presenting cells. A key molecule, DC-SIGN (**d**endritic **c**ell-**s**pecific **I**CAM-3-**g**rabbing **n**onintegrin), also called CD209/DC-SIGN1, is involved in molecular interactions between DC and resting T-cells.¹ DC-SIGN is a type II protein C-type lectin that binds mannose-rich molecules such as ICAM-3 and ICAM-2 in a calcium dependent manner.^{1,2}

DC-SIGN is able to capture HIV-1 through its interaction with the gp120 envelope glycoprotein and promote efficient infection of CD4⁺ T cells that co-express chemokine receptors.³ DC-SIGN, a 44 kDa molecule, is identical to the HIV-1 binding protein.⁴ The spectrum of viruses that interacts with DC-SIGN includes X4, R5, X4R5 HIV-1 strains, HIV-2, and SIV.⁵

Expression of DC-SIGN is induced during the *in vitro* generation of DC from either monocytes or bone marrow progenitors, with maximal surface expression at day 7 of culture.¹ Immature DC in the skin and mature DC in the tonsil have been demonstrated to express DC-SIGN.² Analysis of various tissues and cell lines suggests that DC-SIGN expression is restricted to DC,¹ although there is evidence of expression in placenta, resting monocytes, and monocytic cell lines.⁶

This discrepancy may be partially related to the multiple isoforms of DC-SIGN transcripts, including both membrane and soluble forms, as well as exon splice variants.⁶

Reagent

Supplied as ~500 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline containing 5% trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2–8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of sterile phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody.

Product Profile

This antibody shows inhibition of DC-SIGN expressing NIH-3T3 cell adhesion to ICAM-3 coated 96 well plates. The adhesion of NIH-3T3-DC-SIGN cells (5 x 10⁴ cells/well) to plates coated with 5 µg/mL (100 µL/well) of recombinant human ICAM-3/Fc is maximally inhibited (100%) using 100 µL of a 5 µg/mL antibody solution per well.

Immunohistochemistry: a working concentration of 8–25 µg/mL is recommended using appropriate secondary reagents to detect DC-SIGN in human lymph node in immersion paraffin embedded fixed tissues and tissue sections.

Flow cytometry (immunophenotyping): a stock solution of the monoclonal antibody is added to a concentration of $2.5 \mu\text{g}/10^6$ cells in a minimal volume ($\leq 0.2 \text{ mL}$) of buffer (PBS + 0.5% BSA). The reaction is incubated at room temperature for 30 minutes. The cells are washed two times with the same buffer as above by centrifugation at $250 \times g$ for 5 minutes. The cell pellet is resuspended in 0.2 mL of the PBS buffer and $10 \mu\text{L}$ of a $25 \mu\text{g}/\text{mL}$ of secondary reagent (Goat Anti-Mouse IgG-Fluorescein or Goat Anti-Mouse IgG-Phycoerythrin), is added to the reaction. The cells are incubated for an additional 30 minutes at room temperature and then washed two times as indicated above. The cells are then suspended in 0.5 mL of the same PBS buffer for flow cytometric analysis.

Immunoblotting: a working concentration of $1 \mu\text{g}/\text{mL}$ is recommended using appropriate secondary reagents to detect human DC-SIGN Fc Chimera. Using a colorimetric detection system, the detection limit for recombinant DC-SIGN is approximately $5 \text{ ng}/\text{well}$ under non-reducing and reducing conditions. Using a chemiluminescent detection system will increase sensitivity by 5 to 50 fold.

Immunocytochemistry: a working concentration of $8\text{-}25 \mu\text{g}/\text{mL}$ is recommended using mouse Anti-DC-SIGN-1, and appropriate secondary reagents, in immersion fixed mature human dendritic cells

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

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4. Curtis, B.M., et al., *Proc. Natl. Acad. Sci. USA*, **89**, 8356 (1992).
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6. Mummidi, S., et al., *J. Biol. Chem.*, **276**, 33196-33212 (2001).

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