Monoclonal Anti-human DC-SIGN and DC-SIGNR Antibody-Phycoerythrin

Catalog Number: FAB1621P
Lot Number: LDP02
100 tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse human DC-SIGN and DC-SIGNR: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 120612

Ig class: mouse IgG_{2A}

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing DC-SIGN or DC-SIGNR on their cell surface within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing the DC-SIGN or DC-SIGNR structure. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing DC-SIGN or DC-SIGNR are fluorescently stained, with the intensity of staining directly proportional to the density of these molecules. Cell surface expression of DC-SIGN or DC-SIGNR is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human DC-SIGN and DC-SIGNR: Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization for removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of a rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-DC-SIGN/DC-SIGNR reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-DC-SIGN/DC-SIGNR reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer. (Note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells (in a separate tube) should be treated with PE-labeled mouse IgG_{2A} antibody.

This procedure may need to be modified, depending upon final utilization.

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12/09

DC-SIGN (CD209) and DC-SIGNR (DC-SIGN2)

The recently discovered molecule DC-SIGN (Dendritic Cell- Specific ICAM-3 Grabbing Non-Integrin) has been shown to play an important role in regulating dendritic cell (DC) and T cell interactions, including antigen presentation to T cells and enhancement of transinfection of CD4+ T cells by HIV-1 (1, 2). Efforts to identify additional type II membrane proteins resulted in the isolation of a molecule related in sequence to DC-SIGN known as DC-SIGNR (DC-SIGN Related) (3, 4). DC-SIGNR shares 73 - 80% amino acid homology with DC-SIGN and is located on human chromosome 19p13.3. Its structure is similar to DC-SIGN and therefore binds mannose residues in a calcium dependent fashion, including ICAM-3 and HIV-1 gp120 (5). DC-SIGNR, also known as L-SIGN (Liver/Lymph node-Specific ICAM-3-Grabbing Non-integrin) and DC-SIGNR, is polymorphic since allelic variations of the exon 4 encoded sequence have been isolated (5). This is further supported by a study demonstrating the ability to isolate a large repertoire of DC-SIGNR transcripts largely the result of alternative splicing of the 7 coding exons (6). L-SIGN/DC-SIGNR is primarily transcribed in the liver and lymph nodes but not in monocyte derived DC (5). Expression of L-SIGN/DC-SIGNR is restricted to endothelial cells derived from liver sinusoids, lymph nodes sinuses and capillaries (7) although variable expression in placenta and some monocytic cell lines has also been reported, including both membrane and soluble isoforms of the protein (6).

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 (8). Analysis of various tissues and cell lines suggests that DC-SIGN expression is restricted to DC (1) although a more recent report finds 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 reported in the latter study (6).

1. Geijtenbeek, T.B.H. *et al*. (2000) Cell **100**:575.
2. Geijtenbeek, T.B.H. *et al*. (2000) Cell **100**:587.
3. Yokoyama-Kobayashi, M.T. *et al*. (1999) Gene **228**:161.
4. Soilleux, E.J. *et al*. (2000) J. Immunol. **165**:2937.
5. Bashirova, A.A. *et al*. (2001) J. Exp. Med. **193**:671.
6. Mummidi, S. *et al*. (2001) J. Biol. Chem., 2001 May 3 [epub ahead of print].
7. Pohlman, S. *et al*. (2001) Proc. Natl. Acad. Sci. USA **98**:2670.
8. Geijtenbeek, T.B.H. *et al*. (2000) Nature Immunol. **1**:353.

Figure 1

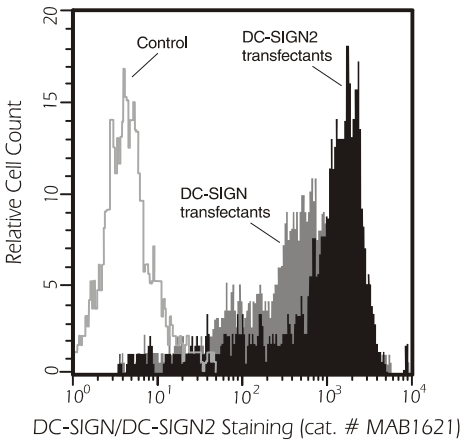

Figure 2A

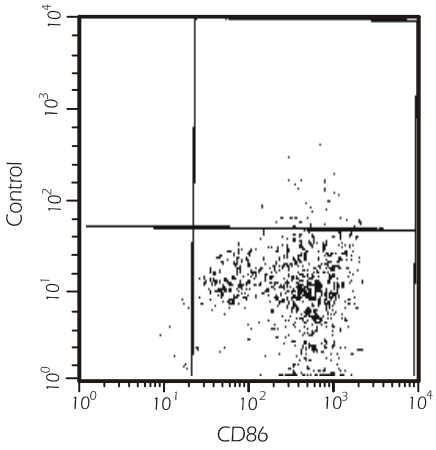

Figure 2B

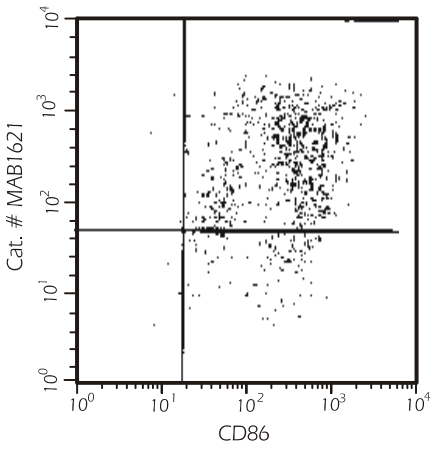

Figure 1
Reactivity of DC-SIGN/DC-SIGNR monoclonal # 120612 (Catalog # MAB1621) with mouse 3T3 cells transfected with either human DC-SIGN or human DC-SIGNR. Control staining indicates reactivity of transfectants with an isotype control reagent.

Figure 2
A. Staining of monocyte derived dendritic cells with CD86-fluorescein and a mouse IgG_{2A} isotype control reagent developed with a goat secondary polyclonal antibody conjugated to phycoerythrin.
B. Staining of the same monocyte derived dendritic cells as above with CD86-fluorescein and anti-human DC-SIGN/DC-SIGNR (Catalog # MAB1621) developed with a goat secondary polyclonal reagent conjugated to phycoerythrin.