Monoclonal Antibodies
Detecting Human Antigens

BD Multicolor
CD86/CD209/CD83

Catalog No. 334098 50 Tests 20 µL/test

Research applications include studies of:

- Phenotyping of monocyte-derived dendritic cell (MDDC) preparations
- Activation and maturation of dendritic cells
- Characterization and immune functions of MDDCs

DESCRIPTION

Specificity

The CD86 antibody recognizes a 75-kilodalton (kDa) cell-surface protein, a second ligand for CD28 and CTLA-4 expressed on T cells.

The CD209 antibody recognizes a dendritic cell–specific ICAM-3–grabbing nonintegrin (DC-SIGN). The CD209 antigen is a type II membrane protein of approximately 44 kDa, with a mannose-binding, C-type lectin domain exclusively expressed by dendritic cells, both in vitro–generated MDDCs and dendritic cells in several tissues, including mucosa and lymph nodes.

The CD83 antibody reacts with a 45-kDa transmembrane protein member of the Ig superfamily and is composed of a single V-type Ig extracellular domain with a C-terminal cytoplasmic tail.

Antigen distribution

CD86 is expressed primarily on dendritic cells, resting monocytes and macrophages, and on activated, but not resting, B lymphocytes, also including large lymphoid cells in germinal centers, Epstein-Barr virus (EBV)–transformed B-cell lines, large B-cell lymphomas, and Reed-Sternberg cells of Hodgkin’s disease. CD86 plays an important role in co-stimulation of T cells in primary immune response and antigen–presenting cell and T-cell interaction.

Reports demonstrate that DC-SIGN binds to a variety of antigens from infectious pathogens such as HIV-1 envelope glycoprotein gp-120, Ebola virus glycoprotein, hepatitis C virus, Dengue virus, and mycobacterium tuberculosis. Reports also suggest that DC-SIGN is most important in the initiation of immune responses by regulating dendritic cell–T-cell interactions. CD209 facilitates dendritic cell migration through endothelium by binding ICAM-2, and mediates the interaction between dendritic cells and resting T cells by binding ICAM-3. DC-SIGN is considered as a new target for designing therapies that block the infections.

CD83 is found mainly on follicular dendritic cells, circulating dendritic cells, interdigitating dendritic cells in lymphoid tissues, and in vitro–generated dendritic cells and thymic dendritic cells. CD83 is a marker of mature dendritic cells. However, its expression is not restricted to dendritic cells. CD83 is also expressed on some germinal...
center B cells and some lymphoblastoid cell lines. Although its function is not known, it might play a role in cell-cell interaction during antigen presentation.\textsuperscript{18,29,30}

**Clones**
- CD86, clone FUN-1, is derived from hybridization of Sp-2 x HBL-1.\textsuperscript{9}
- CD209, clone DCN46, is cloned from a placental library.\textsuperscript{14-17,22}
- CD83, clone HB15e, is derived from Balb/c x NS-1.\textsuperscript{18,29,30}

**Composition**
- CD86 and CD83 are each composed of mouse IgG\textsubscript{1} heavy chains and kappa light chains.
- CD209 is composed of mouse IgG\textsubscript{2b} heavy chains and kappa light chains.

The conjugate is supplied as a combination of CD86 PE, CD209 PerCP-Cy\textsuperscript{TM}5.5, and CD83 APC in 1.0 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA), beta-lactoglobulin, and 0.1\% sodium azide.

**PROCEDURE**
Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

1. Pipet 20 µL of the reagent into a labeled 12 x 75-mm tube.
   Use polypropylene tubes because cell loss can occur when using polystyrene tubes.
2. Add 100 µL of cultured monocyte-derived dendritic cell suspension (4–10 x 10\textsuperscript{5}/mL).\textsuperscript{4}
3. Vortex gently to mix and incubate for 15 to 20 minutes in the dark at room temperature (20° to 25°C).
4. Add 2 mL of wash buffer made with PBS with BSA and sodium azide.
5. Centrifuge at 200 x \textit{g} for 5 minutes; aspirate the supernatant.
6. Resuspend the cell pellet with 0.3 to 0.5 mL of 1\% paraformaldehyde in PBS.
   Keep tubes in the dark for at least 15 minutes, or store in the dark at 4°C for no more than 24 hours.

**REPRESENTATIVE DATA**
Monocyte-derived dendritic cells were prepared according to published protocol.\textsuperscript{31}
Gated on cells with large scatter characteristics (shown as R1). Laser excitation is at 488 nm and 635 nm.
Figure 1  Representative data analyzed with a BD FACS™ brand flow cytometer

Panel A in Figure 1 shows a representative staining profile of 5-day immature MDDC. Panel B in Figure 1 shows 7-day mature MDDC cultured cells derived from monocytes of a normal donor. Each histogram was gated on R1. Instrument settings varied according to sample scatter and fluorescence characteristics of immature and mature MDDC. Dashed lines in the histograms show staining with an isotype control cocktail, and bold lines show staining with MDDC-specific reagents, BD Multicolor CD86 PE/CD209 PerCP-Cy5.5/CD83 APC.

HANDLING AND STORAGE
Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING
All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

CHARACTERIZATION
To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

WARRANTY
Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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REFERENCES


2. Weissman D, Li Y, Ananworanich J, et al. Three populations of cells with dendritic morphology exist in peripheral blood, only one of which is infectable with human immunodeficiency virus type 1. *Proc Natl Acad Sci USA*. 1995;92:826-830.


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