Technical Data Sheet

BB515 Mouse Anti-Human CD209

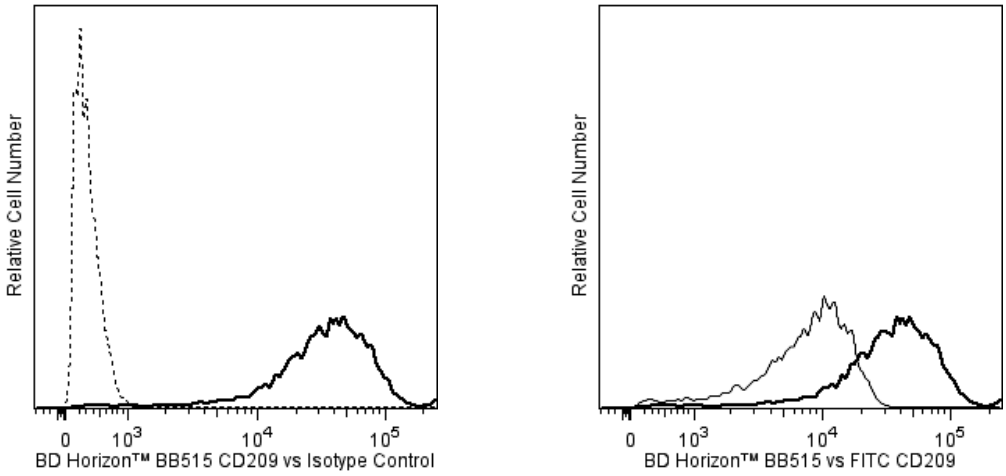
Product Information

Material Number:	**564548**
Alternate Name:	DC-SIGN
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	DCN46
Immunogen:	Human Monocyte Derived DC Cells
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The DCN46 antibody specifically binds to dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN or CD209), a type-II membrane protein of approximately 44 kDa with a mannose-binding C-type lectin domain. It is highly expressed on dendritic cells in mucosal tissues. Its sequence is identical to the HIV-1 envelope gp120-binding C-type lectin, and reports suggest that DC-SIGN binds to HIV-1 gp120 and effectively transmits infectious HIV-1 to resting T lymphocytes expressing CD4 and chemokine receptors. The C-type lectin domain of DC-SIGN is also capable of binding other pathogenic viruses, bacteria, and parasites. Reports also suggest that DC-SIGN enables the highly efficient migration of dendritic cells from blood into the tissues. It can interact with ICAM-2, which has a similar sequence as ICAM-3, and is abundantly expressed on vascular and lymphoid endothelium. Thus, DC-SIGN mediates dendritic cells rolling and transendothelial migration, and its interaction with ICAM-2 is essential to specific migratory functions of dendritic cells.

The antibody was conjugated to BD Horizon BB515 which is part of the BD Horizon Brilliant™ Blue family of dyes. With an Ex Max near 490 nm and an Em Max near 515 nm, BD Horizon BB515 can be excited by the blue laser (488 nm) laser and detected with a 530/30 nm filter. This dye has been exclusively developed by BD Biosciences and is up to seven times brighter than FITC with less spillover into the PE channel. Due to similar excitation and emission properties, BB515, FITC, and Alexa Fluor® 488 cannot be used simultaneously. It is not recommended to use BB515 in cocktails that include Streptavidin conjugates as it may cause high background.



Flow cytometric analysis of CD209 expression on peripheral blood monocyte-derived dendritic cells - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Adherent peripheral blood mononuclear cells were cultured for 7 days with the recombinant human cytokines, GM-CSF (Cat. No. 550068), TNF (Cat. No. 554618), and IL-4 (Cat. No. 554605). The cultured dendritic cells were harvested and stained with either BD Horizon™ BB515 Mouse IgG2b, κ Isotype Control (Cat. No. 564510; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD209 antibody (Cat. No. 564548; bold solid line histogram). Alternatively, cells were stained with FITC Anti-Human CD209 antibody (Cat. No. 551264/561764; thin solid line histogram).*

Overlaid histograms are shown to facilitate staining comparisons between: BB515 Anti-CD209 antibody versus its Ig Isotype Control (Left Panel), and BB515 Anti-CD209 antibody versus FITC Anti-CD209 antibody (Right Panel). The fluorescence histograms showing CD209 expression (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon™ BB515 under optimum conditions and unconjugated antibody was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and CompBead to ensure that BD Comp beads are appropriate for your specific cellular application.

For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescence staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

Catalog Number	Name	Size	Clone
564510	BB515 Mouse IgG2b, κ Isotype Control	50 µg	27-35
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
550068	Recombinant Human GM-CSF	10 µg	(none)
554618	Recombinant Human TNF	10 µg	(none)
554605	Recombinant Human IL-4	5 µg	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Appelmelk BJ, van Die I, van Vliet SJ, et al. Carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J Immunol.* 2003; 170(4):1635-1639. (Biology)

Gruber A, Chalmers AS, Popov S, Ruprecht RM. Functional aspects of binding of monoclonal antibody DCN46 to DC-SIGN on dendritic cells. *Immunol Lett.* 2002; 84(2):103-108. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting, Functional assay)

Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med.* 1995; 182(2):389-400. (Biology)

Steinman RM, Granelli-Piperno A, Pope M, et al. The interaction of immunodeficiency viruses with dendritic cells. *Curr Top Microbiol Immunol.* 2003; 276:1-30. (Biology)

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