Technical Data Sheet

BB515 Mouse Anti-Human CD226

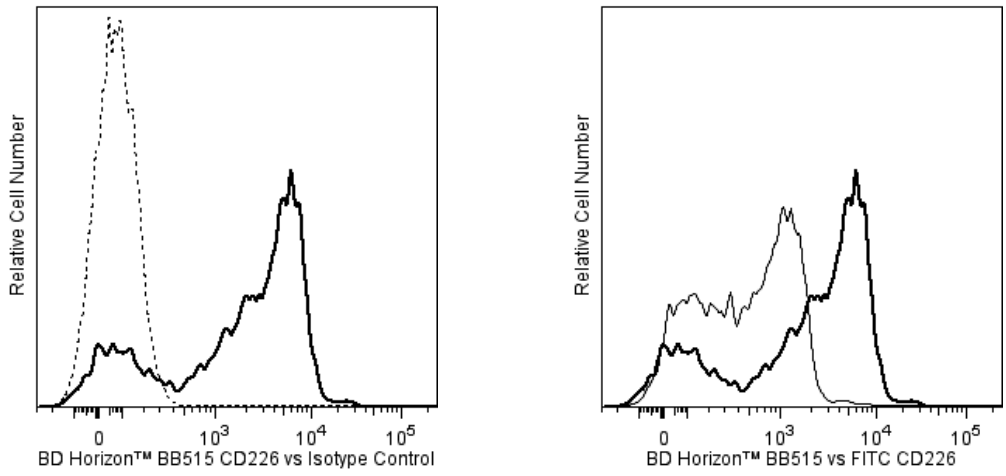
Product Information

Material Number:	**565152**
Alternate Name:	DNAM-1; DNAM1; PTA1; PTA-1; TLiSA1
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	DX11
Immunogen:	Human CTL Clone
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	VII 70648
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The DX11 monoclonal antibody specifically binds to CD226 which is also known as DNAX accessory molecule-1 (DNAM-1), Platelet and T cell activation antigen 1 (PTA1), or T lineage-specific activation antigen 1 antigen (TLiSA1). CD226 is a 65 kDa type 1 transmembrane glycoprotein consisting of 318 amino acid residues including two Ig-like domains. CD226 is expressed on the majority of T cells, NK cells, monocytes, platelets, and a subset of B cells, but not on erythrocytes. It is also present on a subset of thymocytes coexpressing high density surface CD3. CD226 is not present on normal fibroblast cell lines or tumor cell lines of epithelial or neuronal origins. CD226 is a tyrosine phosphorylated, signal-transducing molecule which participates in primary adhesion during cytotoxic T lymphocyte (CTL)- or NK cell-mediated cytotoxicity. The DX11 antibody inhibits T- and NK cell-mediated cytotoxicity against a variety of tumor cell targets, and blocks cytokine production by alloantigen-specific T 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 CD226 expression on human peripheral blood lymphocytes - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human whole blood was depleted of platelets, washed, and preincubated with 5% normal human serum. The cells were then stained with either BD Horizon BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD226 antibody (Cat. No. 565152; bold solid line histogram). Alternatively, cells were stained with FITC Anti-Human CD226 antibody (Cat. No. 559788; thin solid line histogram). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202).*

Overlaid histograms are shown to facilitate staining comparisons between: BB515 Anti-CD226 antibody versus its Ig Isotype Control (Left Panel), and BB515 Anti-CD226 antibody versus FITC Anti-CD226 antibody (Right Panel). The fluorescence histograms showing CD226 expression (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer 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 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).

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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. 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.
6. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
7. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

Lanier LL, Shibuya A, Burns G. CD226 (DNAM-1, PTA1, Tlisa). In: Mason D. David Mason .. et al., ed. *Leucocyte typing VII : white cell differentiation antigens : proceedings of the Seventh International Workshop and Conference held in Harrogate, United Kingdom.* Oxford: Oxford University Press; 2002:921-922. (Clone-specific: Flow cytometry)

Shibuya A, Campbell D, Hannum C, et al. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity.* 1996; 4(6):573-581. (Immunogen: Bioassay, Blocking, Flow cytometry, Functional assay, Immunoaffinity chromatography, Inhibition, Western blot)

Shibuya A, Lanier LL, Phillips JH. Protein kinase C is involved in the regulation of both signaling and adhesion mediated by DNAX accessory molecule-1 receptor. *J Immunol.* 1998; 161(4):1671-1676. (Clone-specific: Bioassay, Blocking, Cytotoxicity, Flow cytometry, Functional assay, Immunoprecipitation)

Zola H, Swart B, Boumsell L, Mason DY. Human Leucocyte Differentiation Antigen nomenclature: update on CD nomenclature. Report of IUIS/WHO Subcommittee. *J Immunol Methods.* 2003; 275(1-2):1-8. (Clone-specific: Flow cytometry)