Technical Data Sheet

BB515 Mouse Anti-Human CD86

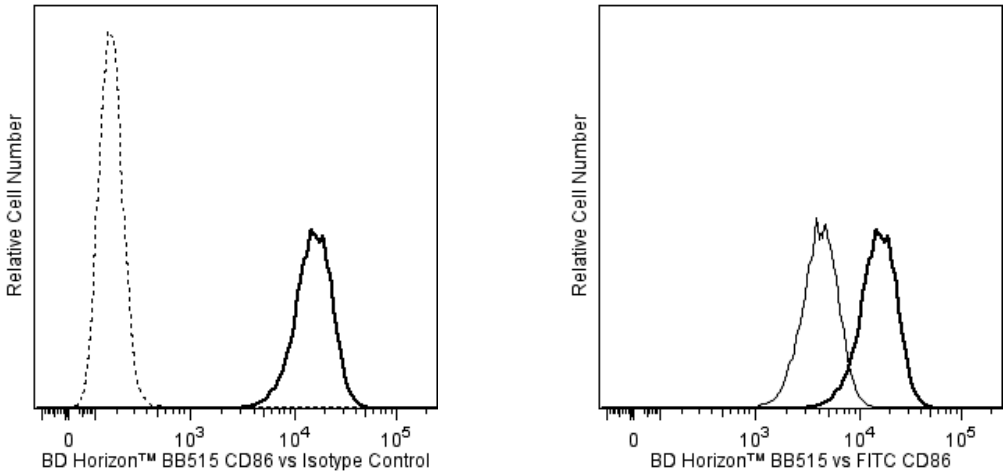
Product Information

Material Number:	**564545**
Alternate Name:	B7.2; B7-2; B-lymphocyte activation antigen B7-2; B70; BU63; CD28LG2; LAB72
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	2331 (FUN-1)
Immunogen:	Human HBL-1 Cell Line
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	V B046, BP126
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2331 (FUN-1) monoclonal antibody specifically recognizes a 75 kDa transmembrane cell surface protein, CD86 (B70/B7-2), expressed primarily on monocytes, dendritic cells and activated B cells. Competitive binding assays demonstrate that, while both 2331 (FUN-1) and IT2.2 (Anti-CD86) antibodies specifically recognize the same molecule, they react with different epitopes. CD86 is a ligand for CD28 and CTLA-4 and plays an important role in costimulation of T cells in primary immune response. The 2331 (FUN-1) antibody blocks the costimulatory activity of CD86 when tested in functional studies.

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 CD86 expression on Daudi cells - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human Daudi B lymphoma cells (ATCC CCL-213) were stained with either BD Horizon BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD86 antibody (Cat. No. 564544/564545; bold solid line histograms). Alternatively, cells were stained with FITC Anti-Human CD86 antibody (Cat. No. 555657/557343/560958; thin solid line histogram).*

Overlaid histograms are shown to facilitate staining comparisons between: BB515 Anti-CD86 antibody versus its Ig Isotype Control (Left Panel), and BB515 Anti-CD86 antibody versus FITC Anti-CD86 antibody (Right Panel). The fluorescence histograms showing CD86 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 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 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
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
564544	BB515 Mouse Anti-Human CD86	100 Tests	2331 (FUN-1)
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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Engel P, Wagner N, Tedder TF. CD86 Workshop Report. In: Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995:703-705. (Clone-specific: Blocking, Flow cytometry, Immunoprecipitation)

Engel P, Gribben JG, Freeman GJ, et al. The B7-2 (B70) costimulatory molecule expressed by monocytes and activated B lymphocytes is the CD86 differentiation antigen. *Blood.* 1994; 84(5):1402-1407. (Clone-specific: Blocking, Enhancement, Flow cytometry, Functional assay, Inhibition)

Nozawa Y, Wachi E, Tominaga K, Abe M, Wakasa H. A novel monoclonal antibody (FUN-1) identifies an activation antigen in cells of the B-cell lineage and Reed-Sternberg cells. *J Pathol.* 1993; 169(3):309-315. (Immunogen: Flow cytometry, Fluorescence microscopy, Immunofluorescence, Immunohistochemistry, Immunoprecipitation)

Nozawa Y, Wakasa H, Abe M. Production and usefulness of monoclonal antibodies against B cells. *Fukushima J Med Sci.* 1999; 45(1):1-11. (Clone-specific)

Nozawa Y, Abe M, Wakasa H. Three mAb, FUN-1, FB1, and FB21, that recognize B-cell antigens in frozen or paraffin-embedded tissue sections. In: Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995:705-706. (Clone-specific: Immunohistochemistry)