Technical Data Sheet

BB515 Mouse Anti-Human CD80

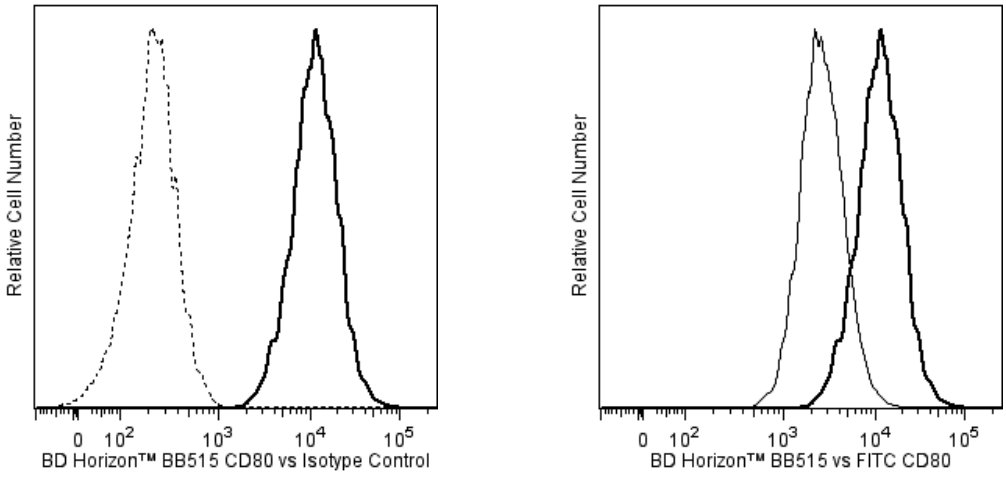
Product Information

Material Number:	**565009**
Alternate Name:	B7.1; B7-1; Activation B7-1 antigen; B7; BB1; CD28LG; CD28LG1; LAB7; L307
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	L307.4 (also known as L307)
Immunogen:	Human B7-transfected L cells
Isotype:	Mouse (C3H) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	V B7.5
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The L307 monoclonal antibody specifically binds to B7/BB1, a 60 kDa transmembrane glycoprotein that was clustered as CD80 in the Fifth International Workshop on Human Leukocyte Differentiation Antigens. CD80, a member of the Ig supergene family, is expressed on activated B cells, T cells, macrophages, and dendritic cells. It is the ligand for two molecules expressed on T cells, CD28 and CD152 (CTLA-4). CD80 is also expressed on activated CD4-positive and CD8-positive T cells, appearing late after activation suggesting that activated T cells may be capable of autocrine costimulation via the CD28 activation pathway. The binding of CD28 by anti-CD28 or by CD80 results in T-cell activation and a signal for IL-2 production.

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 CD80 expression on human Raji cells - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Cells from the human Raji (Burkitt's B cell lymphoma, ATCC CCL-86) cell line 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 CD80 antibody (Cat. No. 565008/565009; bold solid line histograms). Alternatively, cells were stained with FITC Anti-Human CD80 antibody (Cat. No. 557226/560926; thin solid line histogram). Overlaid histograms are shown to facilitate staining comparisons between: BB515 Mouse Anti-Human CD80 antibody versus its Ig Isotype Control (Left Panel), and BB515 Mouse Anti-Human CD80 antibody versus FITC Mouse Anti-Human CD80 antibody (Right Panel). The fluorescence histograms showing CD80 expression (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable Raji cells. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

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
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 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

Azuma M, Yssel H, Phillips JH, Spits H, Lanier LL. Functional expression of B7/BB1 on activated T lymphocytes. *J Exp Med.* 1993; 177(3):845-850. (Immunogen: Flow cytometry, Immunoprecipitation, Induction)

Koulova L, Clark EA, Shu G, Dupont B. The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of CD4+ T cells. *J Exp Med.* 1991; 173(3):759-762. (Biology)

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(Clone-specific: Flow cytometry)

Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell.* 1992; 71(7):1065-1068. (Biology)