Technical Data Sheet

BB515 Mouse Anti-Human HLA-DR

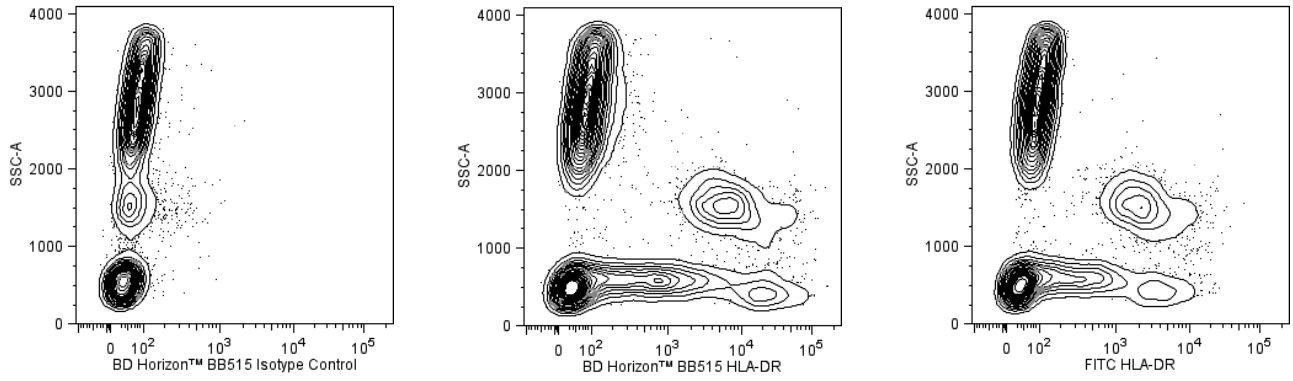
Product Information

Material Number:	**564516**
Alternate Name:	MHC class II antigen; HLA class II histocompatibility antigen
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	G46-6
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon Reported Reactivity: Dog
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The G46-6 monoclonal antibody specifically binds to HLA-DR, a major histocompatibility complex (MHC) class II antigen. HLA-DR antigens are encoded by genes within the Human Leukocyte Antigen (HLA) Complex located on chromosome 6. HLA-DR is a transmembrane heterodimeric glycoprotein composed of an α chain (36 kDa) and a β subunit (27 kDa) expressed primarily on antigen presenting cells: B cells, dendritic cells, monocytes, macrophages, and thymic epithelial cells. HLA-DR is also expressed on activated T cells. This molecule plays a major role in mediating cellular interactions during antigen presentation to CD4-positive 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.



Two-parameter flow cytometric analysis of HLA-DR expression on human peripheral blood leucocytes - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human whole blood was stained with either BD Horizon BB515 Mouse IgG2a, κ Isotype Control (Cat. No. 564515; Left Panel) or BD Horizon BB515 Mouse Anti-Human HLA-DR antibody (Cat. No . 564516; Middle Panel). Alternatively, cells were stained with FITC Anti-Human HLA-DR antibody (Cat. No . 555811/556643/560944; Right Panel). The erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). Two-parameter flow cytometric contour plots showing the correlated expression of HLA-DR (or Ig Isotype control staining) versus side-light scatter (SSC-A) signals were derived from gated events with the forward and side-light scatter characteristics of intact leucocyte populations. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.*

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.

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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)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
564515	BB515 Mouse IgG2a, κ Isotype Control	50 µg	G155-178
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. 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

Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. *J Exp Med.* 2001; 193(11):1303-1310. (Clone-specific: Flow cytometry)

Ibisch C, Pradal G, Bach JM, Lieubeau B. Functional canine dendritic cells can be generated in vitro from peripheral blood mononuclear cells and contain a cytoplasmic ultrastructural marker. *J Immunol Methods.* 2005; 298(1-2):175-82. (Clone-specific)

Kitani A, Chua K, Nakamura K, Strober W. Activated self-MHC-reactive T cells have the cytokine phenotype of Th3/T regulatory cell 1 T cells. *J Immunol.* 2000; 165(2):691-702. (Clone-specific: Flow cytometry)

Moran TP, Collier M, McKinnon KP, Davis NL, Johnston RE, Serody JS. A novel viral system for generating antigen-specific T cells. *J Immunol.* 2008; 175(5):3431-3438. (Clone-specific: Flow cytometry)

Sorg RV, Kogler G, Wernet P. Identification of cord blood dendritic cells as an immature CD11c- population. *Blood.* 1999; 93(7):2302-2307. (Clone-specific: Flow cytometry)