Technical Data Sheet

BB515 Mouse Anti-Human HLA-A2

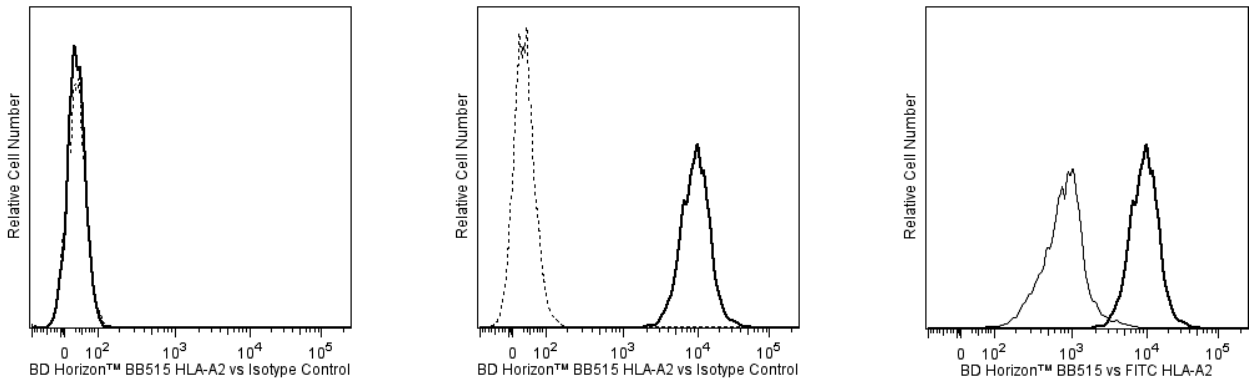
Product Information

Material Number:	**565930**
Alternate Name:	HLA class I histocompatibility antigen A2 alpha chain; HLA-A2
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	BB7.2
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The monoclonal antibody BB7.2 specifically binds to the α subunit of the human leukocyte antigen-A2 (HLA-A2), a class I molecule of the major histocompatibility complex (MHC). The MHC gene locus encodes a group of highly polymorphic, cell-surface proteins that play a broad role in the immune response to protein antigens. MHC molecules bind and present small antigenic protein fragments to antigen-specific receptors expressed by T cells (TCR). Human (*human leukocyte antigen*/HLA) MHC molecules are comprised of two major classes, MHC class I and class II. Functionally, class I MHC molecules bind peptides derived from intracellular antigens (eg, viral and some bacterial antigens) which are specifically recognized by CD8+ T cells. Class II MHC molecules bind antigens derived from pathogens multiplying in intracellular vesicles and ingested extracellular bacteria, both of which are recognized by CD4+ T cells. TCR recognize processed peptides bound to the MHC as well as regions of the MHC molecule itself. CD4 and CD8 accessory molecules strengthen the formation of the TCR-MHC complex through their interaction with non-polymorphic regions of the MHC molecule.

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 human HLA-A2 expression on lymphocytes from HLA-A2-positive and -negative donors - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. *Human whole blood was obtained from either an HLA-A2-negative (Left Panel) or an HLA-A2-positive (Middle and Right Panels) donor. The blood was stained with either BD Horizon™ BB515 Mouse IgG2b, κ Isotype Control (Cat. No. 564510; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human HLA-A2 antibody (Cat. No. 564577/565930; bold solid line histogram). Alternatively, cells were stained with FITC Anti-Human HLA-A2 antibody (Cat. No. 551285/ 561107; thin solid line histogram). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202).*

Overlaid histograms are shown to facilitate staining comparisons between: BB515 Anti-HLA-A2 antibody versus its Ig Isotype Control (Left Panel and Middle Panels), and BB515 Anti-HLA-A2 antibody versus FITC Anti-HLA-A2 antibody (for HLA-A2-positive donor cells; Right Panel). The fluorescence histograms showing HLA-A2 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™ 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 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
564577	BB515 Mouse Anti-Human HLA-A2	50 µg	BB7.2
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
564510	BB515 Mouse IgG2b, κ Isotype Control	50 µg	27-35
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. 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. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
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/us/s/resources for technical protocols.

References

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature.* 1987; 329(6139):506-512. (Biology)

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature.* 1987; 329(6139):512-518. (Biology)

Parham P, Brodsky FM. Partial purification and some properties of BB7.2. A cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. *Hum Immunol.* 1981; 3(4):277-299. (Clone-specific)

Romero P, Dunbar PR, Valmori D. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. *J Exp Med.* 1998; 188(9):1641-1650. (Biology)