

Technical Data Sheet

BB515 Mouse Anti-Human CD45

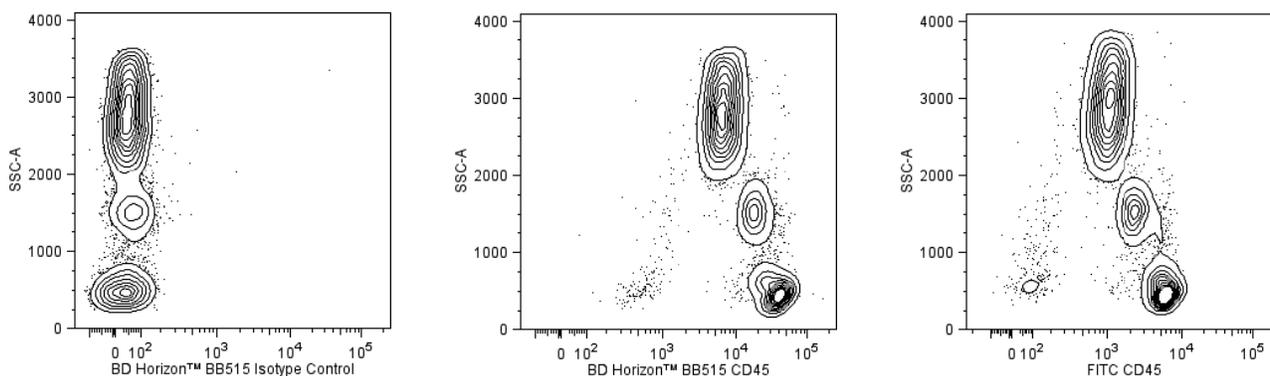
Product Information

Material Number:	564585
Alternate Name:	PTPRC; LCA; L-CA; Leukocyte Common Antigen; T200; GP180; LY5
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	HI30
Immunogen:	Human Peripheral Blood Leucocytes
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	IV N816
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The HI30 monoclonal antibody specifically binds to the 180, 190, 205, 220 kDa protein isoforms of CD45. CD45 is encoded by the *PTPRC* (Protein tyrosine phosphatase receptor type C) gene. CD45, also known as the leukocyte common antigen (LCA), is a member of the protein tyrosine phosphatase (PTP) family. It is present on all human leukocytes including lymphocytes, monocytes, granulocytes, eosinophils, and thymocytes. CD45 is absent from circulating erythrocytes, platelets, or mature erythroid cells of bone marrow and non-hemopoietic tissues.

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 CD45 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 IgG1, κ Isotype Control (Cat. No. 564416; Left Panel) or BD Horizon BB515 Mouse Anti-Human CD45 antibody (Cat. No. 564585/564586; Middle Panel). Alternatively, cells were stained with FITC Anti-Human CD45 antibody (Cat. No. 555482/ 560976/561865; Right Panel). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). Two-parameter flow cytometric contour plots showing the correlated expression of CD45 (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™ LSR II Flow Cytometer 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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564585 Rev. 4



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
564586	BB515 Mouse Anti-Human CD45	25 Tests	HI30
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)
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 www.regdocs.bd.com to access safety data sheets (SDS).
7. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

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