

Technical Data Sheet

BB515 Rat Anti-Mouse CD5

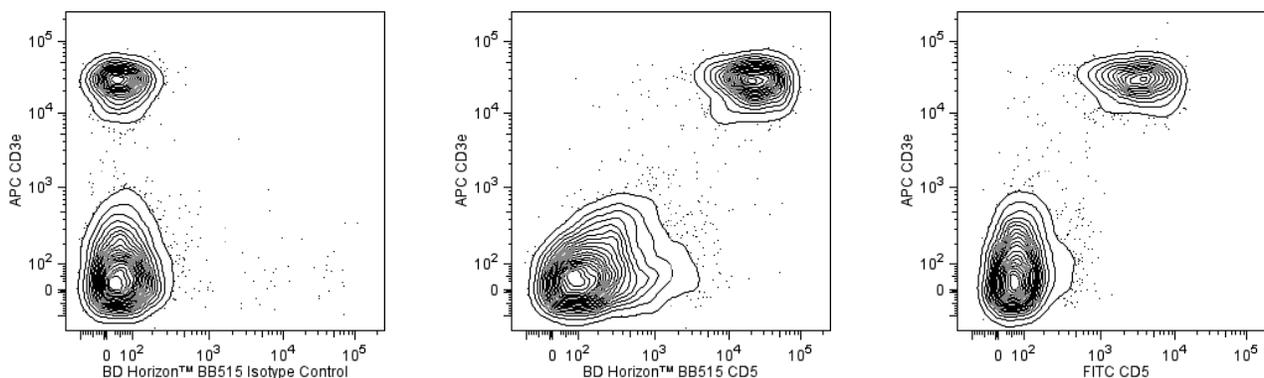
Product Information

| | |
|-------------------------|---|
| Material Number: | 565504 |
| Alternate Name: | Lymphocyte antigen 1; Cd5; Ly-12; Ly-A; Lyt-1 |
| Size: | 50 µg |
| Concentration: | 0.2 mg/ml |
| Clone: | 53-7.3 |
| Immunogen: | Mouse Thymus / Spleen |
| Isotype: | Rat (LOU) IgG2a, κ |
| Reactivity: | QC Testing: Mouse |
| Storage Buffer: | Aqueous buffered solution containing ≤0.09% sodium azide. |

Description

The 53-7.3 monoclonal antibody specifically binds to a monomorphic epitope of CD5, a member of the scavenger receptor cysteine-rich protein superfamily and the major ligand of CD72, found on thymocytes, T lymphocytes, thymic NKT cells, and a subset of B lymphocytes, but not on NK cells or splenic NKT cells. The level of surface CD5 expression is developmentally regulated in the thymus, starting with low levels on CD4-CD8- thymocytes and increasing as they mature to CD4+CD8+ then CD4+CD8- or CD4-CD8+ thymocytes. Relatively high levels are maintained on peripheral T lymphocytes. The level of CD5 antigen detected on T helper cells has been reported to be somewhat higher than that on T cytotoxic/suppressor and B cells. Few, if any, intestinal intraepithelial lymphocytes bearing the $\gamma\delta$ T-cell receptor express CD5. Phenotypic, anatomical, functional, developmental, and pathogenic characteristics of peripheral CD5+ B cells suggest that they may represent a distinct lineage, known as B-1 cells. The frequency of these CD5+ B cells has been reported to show strain-dependent variation. An additional population of CD5+ B lymphocytes resides in the thymus, where it matures from intrathymic B-cell progenitors. It has been proposed that CD5 is a costimulatory molecule which mediates interactions of cells in the immune system and negatively regulates signal transduction mediated by the T-cell receptor and B-cell receptor.

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-color flow cytometric analysis of CD5 expression on mouse splenocytes - Staining comparisons between BD Horizon™ BB515- and FITC-conjugated antibodies. Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with APC Hamster Anti-Mouse CD3e antibody (Cat. No. 553066/561826) and either BD Horizon BB515 Rat IgG2a, κ Isotype Control (Cat. No. 564418; Left Panel), BD Horizon BB515 Rat Anti-Mouse CD5 antibody (Cat. No. 565504; Middle Panel), or FITC Rat Anti-Mouse CD5 antibody (Cat. No. 553020/553021; Right Panel). Two-color flow cytometric contour plots showing the correlated expression of CD5 (or Ig Isotype control staining) versus CD3e were derived from gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. 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.

Application Notes

Application

| | |
|----------------|------------------|
| Flow cytometry | Routinely Tested |
|----------------|------------------|

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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 |
|----------------|------------------------------------|------------|----------|
| 554656 | Stain Buffer (FBS) | 500 mL | (none) |
| 554657 | Stain Buffer (BSA) | 500 mL | (none) |
| 563794 | Brilliant Stain Buffer | 100 Tests | (none) |
| 564418 | BB515 Rat IgG2a, κ Isotype Control | 0.1 mg | R35-95 |
| 553066 | APC Hamster Anti-Mouse CD3e | 0.1 mg | 145-2C11 |
| 561826 | APC Hamster Anti-Mouse CD3e | 25 µg | 145-2C11 |
| 555899 | Lysing Buffer | 100 mL | (none) |
| 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. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
6. 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.
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

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