

Technical Data Sheet

BB515 Mouse Anti-Human CD25

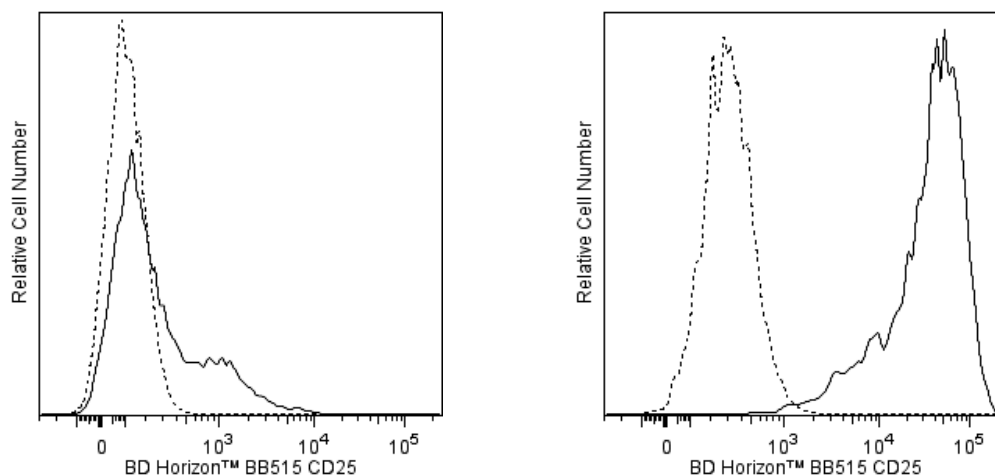
Product Information

Material Number:	565096
Alternate Name:	IL-2R; IL2RA; IL-2R α ; TCGFR; TAC antigen; p55
Size:	100 Tests
Vol. per Test:	5 μ l
Clone:	M-A251
Immunogen:	Phytohemagglutinin stimulated human lymphocytes
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	IV A053
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The M-A251 monoclonal antibody specifically binds to the 55 kDa type I transmembrane glycoprotein known as low-affinity interleukin-2 receptor alpha chain subunit (IL-2R α). CD25 is expressed on regulatory T cells, activated lymphocytes (T and B), and monocytes. It associates with the IL-2R β /CD122 and IL-2R γ /CD132 receptor chains to form the high-affinity IL-2R complex. CD25 expression on T and B lymphocytes is upregulated by antigenic or mitogenic stimulation. Soluble CD25/IL-2R α is produced as a consequence of lymphocyte stimulation and is found in biological fluids following inflammatory responses.

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 CD25 expression on unstimulated and stimulated human lymphocytes.

Left Panel: Whole blood was stained with either BD Horizon™ BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; dashed line histogram) or BD Horizon BB515 Mouse Anti-Human CD25 antibody (Cat. No. 565096; solid line histogram). The erythrocytes were subsequently lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899).

Right Panel: Phytohemagglutinin-stimulated (3 days) human peripheral blood mononuclear cells were stained with either BD Horizon BB515 Mouse Anti-Human CD25 antibody (solid line histogram) or with BD Horizon BB515 Mouse IgG1, κ Isotype Control (dashed line histogram).

The fluorescence histograms showing CD25 expression (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes (Left Panel) or lymphoblasts (Right Panel). Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

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565096 Rev. 3



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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 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. 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 <http://regdocs.bd.com> to access safety data sheets (SDS).
8. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

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