Technical Data Sheet

BB515 Rat Anti-Mouse CD25

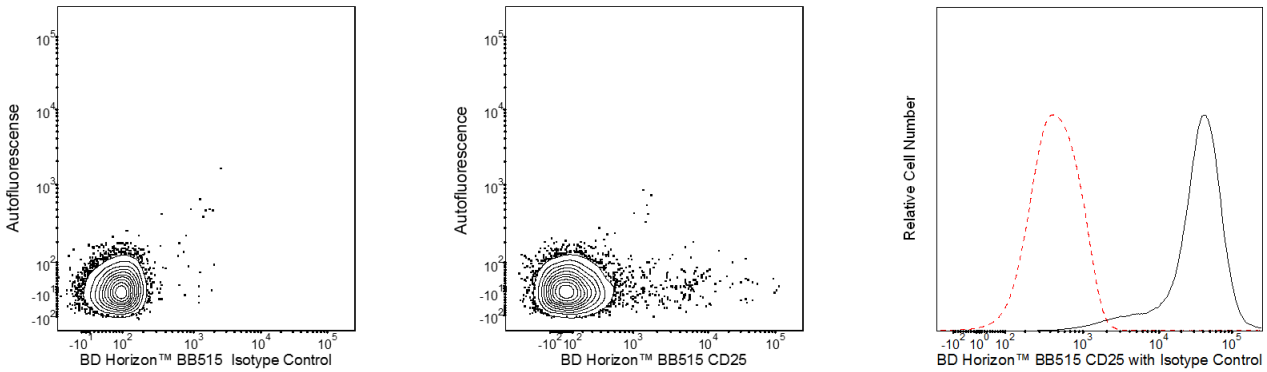
Product Information

Material Number:	**564458**
Alternate Name:	Interleukin-2 receptor alpha chain; IL-2RA; IL-2Rα; Il2ra; IL-2R p55
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	PC61
Immunogen:	IL-2-dependent cytolytic mouse T-cell clone B6.1
Isotype:	Rat (OFA) IgG1, λ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The PC61 monoclonal antibody specifically binds to CD25, the low-affinity IL-2 Receptor α chain (IL-2Rα, p55) expressed on activated T and B lymphocytes from all mouse strains tested. IL-2Rα by itself is not a signaling receptor. However, it can combine with IL-2 Receptor β (CD122) and γc (CD132) chains to form high-affinity, signaling receptor complexes for IL-2. Resting T and B lymphocytes and resting and activated NK cells do not express IL-2Rα. CD25 is transiently expressed at a low level during normal B-cell development in the bone marrow on the CD45R/B220low TdT- sIg- Pre-B/Pre-B-II and CD45R/B220low TdT- sIgM+ sIgD- immature B stages, but not on the CD45R/B220low TdT+ sIg- Pro-B/Pre-B-I stage nor on CD45R/B220high TdT- sIgM+ sIgD+ mature B cells. It is expressed at a higher level during a very early stage of T-cell development in fetal and adult thymus. Peripheral CD25+CD4+ lymphocytes called regulatory T (Treg) cells are involved in the maintenance of self-tolerance. It has also been reported that dendritic cells express CD25, recognized by mAb 7D4. The PC61 antibody recognizes an epitope of CD25 which is distinct from the IL-2 binding site and from those recognized by mAbs 3C7 and 7D4. It blocks binding of IL-2 to CD25, presumably by inducing a conformational change in CD25.

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 mouse splenocytes.

Left and Middle Panels: *Freshly prepared 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 either BD Horizon™ BB515 Rat IgG1, λ Isotype Control (Cat. No. 564417; Left Panel) or BD Horizon™ BB515 Rat Anti-Mouse CD25 antibody (Cat. No. 564424/564458; Middle Panel). Two-color flow cytometric contour plots showing the correlated expression patterns for Ig Isotype control staining (Left Panel) or CD25 expression (Middle Panel) versus cellular autofluorescence (measured in the APC/allophycocyanin channel) were generated for gated events with the forward and side light- scatter characteristics of viable lymphocytes.*

Right Panel: *Mouse splenic leucocytes were stimulated with concanavalin A for 3 days. The cells were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™). The cells were then stained with either BD Horizon™ BB515 Rat IgG1, λ Isotype Control (dashed line histogram) or BD Horizon™ BB515 Rat Anti-Mouse CD25 antibody (solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphoblasts.*

Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

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 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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564417	BB515 Rat IgG1, λ Isotype Control	0.1 mg	A110-1
564424	BB515 Rat Anti-Mouse CD25	100 µg	PC61
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
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. 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. 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.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Ceredig R, Lowenthal JW, Nabholz M, MacDonald HR. Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells. *Nature.* 1985; 314(6006):98-100. (Clone-specific: Blocking, Immunohistochemistry)

Chen J, Ma A, Young F, Alt FW. IL-2 receptor alpha chain expression during early B lymphocyte differentiation. *Int Immunol.* 1994; 6(8):1265-1268. (Biology)

Ernst DN, Weigle WO, McQuitty DN, Rothermel AL, Hobbs MV. Stimulation of murine T cell subsets with anti-CD3 antibody. Age-related defects in the expression of early activation molecules. *J Immunol.* 1989; 142(5):1413-1421. (Clone-specific: Flow cytometry)

Garni-Wagner BA, Witte PL, Tutt MM, et al. Natural killer cells in the thymus. Studies in mice with severe combined immune deficiency. *J Immunol.* 1990; 144(3):796-803. (Biology)

Godfrey DI, Zlotnik A. Control points in early T-cell development. *Immunol Today.* 1993; 14(11):547-553. (Biology)

Lowenthal JW, Corthésy P, Tougne C, Lees R, MacDonald HR, Nabholz M. High and low affinity IL 2 receptors: analysis by IL 2 dissociation rate and reactivity with monoclonal anti-receptor antibody PC61. *J Immunol.* 1985; 135(6):3988-3994. (Immunogen: Bioassay, Blocking, Inhibition, Radioimmunoassay)

Lowenthal JW, Zubler RH, Nabholz M, MacDonald HR. Similarities between interleukin-2 receptor number and affinity on activated B and T lymphocytes. *Nature.* 1985; 315(6021):669-672. (Clone-specific: Blocking, Immunoprecipitation, Radioimmunoassay)

Moreau JL, Nabholz M, Diamantstein T, Malek T, Shevach E, Theze J. Monoclonal antibodies identify three epitope clusters on the mouse p55 subunit of the interleukin 2 receptor: relationship to the interleukin 2-binding site. *Eur J Immunol.* 1987; 17(7):929-935. (Clone-specific: Blocking)

Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med.* 2000; 192(2):295-302. (Biology)