Technical Data Sheet

BV421 Mouse Anti-Human CD25

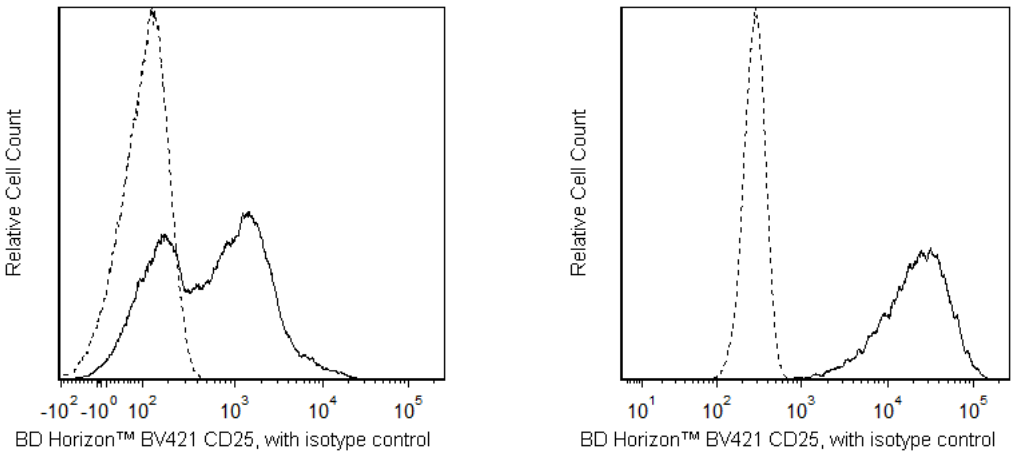
Product Information

Material Number:	**562443**
Alternate Name:	IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55
Size:	25 Tests
Vol. per Test:	5 µl
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 BSA and ≤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-2Rα). CD25 is expressed on regulatory T cells, activated lymphocytes (T and B), and monocytes. It associates with the IL-2Rβ/CD122 and IL-2Rγ/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-2Rα is produced as a consequence of lymphocyte stimulation and is found in biological fluids following inflammatory responses.

The antibody was conjugated to BD Horizon BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max near 407 nm and Em Max near 421 nm, BD Horizon BV421 can be excited by the violet laser (405 nm) and detected with a 450/50 nm filter. BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific BlueTM conjugates. Due to nearly identical excitation and emission properties but different spillover characteristics, BD Horizon BV421, Pacific Blue, and BD Horizon V450 cannot be used simultaneously.



Flow cytometric analysis of CD25 expression on unstimulated and stimulated human peripheral blood lymphocytes.

Left Panel: Whole blood was stained with either BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438; dashed line histogram) or BD Horizon™ BV421 Mouse Anti-Human CD25 antibody (Cat. No. 562442/562443; 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™ BV421 Mouse Anti-Human CD25 antibody (solid line histogram) or with BD Horizon™ BV421 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 FACSCanto™ 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 BV421 under optimum conditions, and unconjugated antibody and free BD Horizon BV421 were 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).

Suggested Companion Products

Catalog Number	Name	Size	Clone
562442	BV421 Mouse Anti-Human CD25	100 Tests	M-A251
562438	BV421 Mouse IgG1, k Isotype Control	50 µg	X40
554656	Stain Buffer (FBS)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
8. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 8,575,303; 8,354,239.
9. 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.
10. Please refer to www.regdocs.bd.com to access safety data sheets (SDS).
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Knapp W. W. Knapp .. et al., ed. *Leucocyte typing IV : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1989:1-1182. (Clone-specific)
Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)