Technical Data Sheet

BV650 Mouse Anti-Human CD25

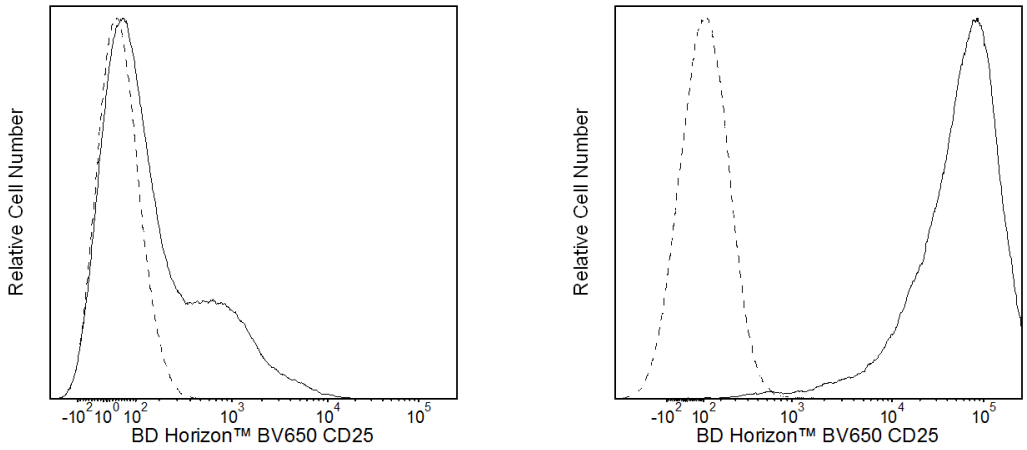
Product Information

Material Number:	**563718**
Alternate Name:	IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55
Size:	25 tests
Vol. per Test:	5 µl
Clone:	M-A251
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
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 the low-affinity interleukin-2 receptor alpha chain subunit (IL-2Rα). CD25 is expressed on regulatory T cells and on activated lymphocytes (T and B) and monocytes. It associates with the IL-2Rβ/CD122 and the 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™ BV650 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon™ BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



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

Left Panel: Whole blood was stained with either BD Horizon™ BV650 Mouse IgG1, κ Isotype Control (Cat. No. 563231; dashed line histogram) or BD Horizon™ BV650 Mouse Anti-Human CD25 antibody (Cat. No. 563718/563719; 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™ BV650 Mouse Anti-Human CD25 antibody (solid line histogram) or with BD Horizon™ BV650 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.

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™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

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Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563231	BV650 Mouse IgG1, k Isotype Control	50 µg	X40
555899	Lysing Buffer	100 ml	(none)
349202	BD FACS™ Lysing Solution	100 ml	(none)
563719	BV650 Mouse Anti-Human CD25	100 tests	M-A251

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Brilliant Violet™ 650 is a trademark of Sirigen.

References

Janszen M, Buck D, Maino VC. Functional and molecular properties of CD25 monoclonal antibodies. In: Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:403-406. (Clone-specific: Blocking, Flow cytometry)

Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV.* New York, NY: Oxford University Press; 1989:1-1182. (Clone-specific)

Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. TGF-β1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. *J Immunol.* 2004; 172(2):834-842. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting)

Ravoet AM, Latinne D, Couvreur B, Zenebergh A, De Bruyère, Ninane J, Sokal G. CD25 mab: Epitopes recognized, effect on lymphocyte activation, mediation of ADCC. In: Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:408-411. (Clone-specific: Functional assay)

Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens.* New York: Oxford University Press; 1995. (Biology)

Schwarting R, Stein H. Cluster report: CD25. In: Knapp W, Dörken B, Gilks WR, et al, ed. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:399-403. (Clone-specific: Flow cytometry, Immunoprecipitation)

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