Technical Data Sheet

BV605 Mouse Anti-Human CD25

Product Information

Material Number:
Alternate Name:
Size:
Vol. per Test:
Clone:
Immunogen:
Isotype:
Reactivity:
Workshop:
Storage Buffer:

562660 IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55 100 Tests 5 µl/test 2A3 Human Phytohemagglutinin-activated T Cells Mouse (BALB/c) IgG1, κ QC Testing: Human III A769,T153; IV A8 Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 2A3 monoclonal antibody specifically binds to human CD25, the low-affinity alpha subunit of the Interleukin-2 Receptor (IL- $2R\alpha$). CD25 associates with CD122 (IL- $2R\beta$ chain) and CD132 (common γ chain or γ c) to form the high-affinity signal-transducing IL-2R complex. CD25 is expressed by subsets of thymocytes and peripheral blood lymphocytes including CD4+CD25+ regulatory T cells and memory T cells. CD25 antigen density increases on activated T cells including phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes. High levels of CD25 can be expressed by T lymphocytes from mixed lymphocyte cultures and by human T-lymphocyte leukemia virus (HTLV)-infected T-lymphocyte leukemia lines, for example, HUT-102. CD25 can also be expressed by activated B cells and macrophages. Recombinant IL-2 blocks the binding of the 2A3 antibody to PHA-activated T lymphocytes.

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant[™] Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

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).



Flow cytometric analysis of CD25 expression on stimulated human peripheral blood lymphocytes. Phytohemagglutinin-stimulated (3 days) peripheral blood mononuclear cells were stained with BD HorizonTM BV605 Mouse Anti-Human CD25 antibody (Cat. No. 562660/562661; solid line histogram) or with BD HorizonTM BV605 Mouse IgG1, κ Isotype Control (Cat. No. 562652; dotted line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphoblasts. Flow cytometric analysis was performed using a BDTM LSR 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 HorizonTM BV605 under optimum conditions, and unconjugated antibody and free BD HorizonTM

BV605 were removed. **Application Notes**

App	licat	tion	

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Flow cytometry	r cytometry Routinely Tested					
Suggested Companion Products						
Catalog Number	Name	Size	Clone			
562652	BV605 Mouse IgG1, κ Isotype Control	50 μg	X40			
554656	Stain Buffer (FBS)	500 mL	(none)			
562661	BV605 Mouse Anti-Human CD25	25 Tests	2A3			
563794	Brilliant Stain Buffer	5 mL	(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).
- An isotype control should be used at the same concentration as the antibody of interest. 2
- Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the 3 residual emission from BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon[™] BV605 conjugate.
- 4. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR. 5.
- 6. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 7.
- 8. 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.
- 9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- 10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 11. CF[™] is a trademark of Biotium, Inc.

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