Technical Data Sheet

PE-Cy™7 Mouse Anti-Human CD25

Product Information

Material Number:	561405
Alternate Name:	IL-2R; IL2RA; IL-2Rα; TCGFR; TAC antigen; p55
Size:	50 tests
Vol. per Test:	5 μl
Clone:	M-A251
Isotype:	Mouse IgG1, ĸ
Reactivity:	Human
	QC Testing: Rhesus or Cynomolgus Macaques or Baboons
Workshop:	IV A053
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ 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-2Ra). CD25 is expressed on regulatory T cells and on activated lymphocytes (T and B) and monocytes. It associates with the IL-2R \$\beta/CD122\$ and the IL-2R \$\psi/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-2Ra is produced as a consequence of lymphocyte stimulation and is found in biological fluids following inflammatory responses.



Flow cytometric analysis of CD25 expression on stimulated Rhesus macaque peripheral blood lymphocytes. Phytohemagglutinin-stimulated (3 days) peripheral blood mononuclear cells from a Rhesus macaque donor were stained with either PE-Cy™7 Mouse Anti-Human CD25 (Cat. No. 561405; solid line histogram), or with a PE-Cy™7 Mouse IgG1, κ Isotype Control (Cat. No. 557872; dashed line histogram). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphoblast cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer Svstem.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application						
Flow cytometry	Routinely Tested					
Suggested Compa	nion Products					
Catalog Number	Name		Size	Clone		
557872	PE-Cy™7 Mouse IgG1 к Isotype Control		100 tests	MOPC-21		
554656	Stain Buffer (FBS)		500 ml	(none)		
BD Biosciences						
bdbiosciences.com						
United States Canada 877.232.8995 888.268.543	Europe Japan 30 32.53.720.550 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 0800.771.7157		B	
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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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
- 6. 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.
- 7. 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.
- 8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD[™] Stabilizing Fixative (Cat. No. 338036).
- 10. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
- 11. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook*. San Diego, CA: Academic Press; 1997. (Biology) Knapp W, Dorken B, Rieber EP, et al, ed. *Leucocyte Typing IV*. New York: Oxford University Press; 1989:1-1208. (Clone-specific) Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens*. New York: Oxford University Press; 1995. (Biology)