Technical Data Sheet

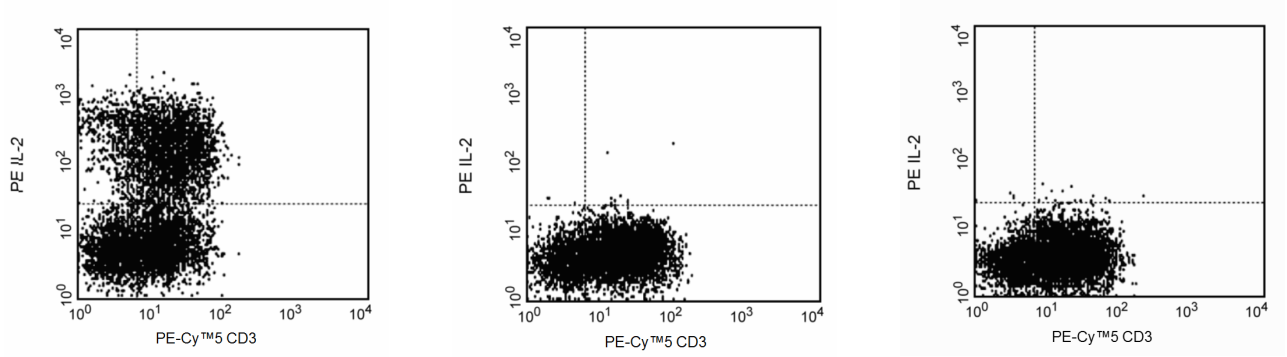
PE Rat Anti-Human IL-2

Product Information

Material Number:	**559334**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	MQ1-17H12
Immunogen:	Human IL-2 Recombinant Protein
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MQ1-17H12 antibody reacts with human interleukin-2 (IL-2). The immunogen used to generate the MQ1-17H12 hybridoma was recombinant human IL-2. Unconjugated or purified forms of this antibody have been reported to be neutralizing for human IL-2 bioactivity.



Expression of IL-2 by stimulated CD3+ and CD3-human PBMC. *Human PBMCs were stimulated for 18 hours with PMA (Sigma, Cat. No. P-8139) and ionomycin (Sigma, Cat. No. I-0634), in the presence of BD GolgiStop™ (2 µl final concentration; Cat. No. 554724). The PBMCs were stained with PE-Cy™5-anti-CD3 (Cat. No. 555334), fixed, permeabilized, and then stained with 20 µl of PE-Rat anti-Human IL-2 antibody (Cat. No. 559334; left panel). To demonstrate specificity of staining, the binding of PE-MQ1-17H12 was blocked by the preincubation of the conjugated antibody with recombinant human IL-2 (0.25 mg, Cat. No. 554603; middle panel), and by preincubation of the fixed/permeabilized cells with an excess of the unlabeled MQ1-17H12 antibody (10 mg, Cat. No. 554563; right panel) prior to staining with the PE-MQ1-17H12 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) and unlabeled antibody blocking specificity controls (right panel).*

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated MQ1-17H12 antibody (Cat. No. 559334) can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-2-producing cells within mixed cell populations (see image). This 100 Test Size formulation of the PE-conjugated MQ1-17H12 antibody has been pre-titrated to assure effective intracellular detection of human IL-2 using 20 µl/1 × 10^6 cells.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MQ1-17H12 antibody with a molar excess of ligand (e.g., recombinant human IL-2; MQ1-17H12 antibody (Cat. No. 554563) prior to staining. A suitable rat IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-R35-95 (Cat. No. 559317). For specific methodology, please visit the protocols section on our web site, http://www.bdbiosciences.com/resources/index.jsp

Important Note: This pretitered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization

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agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. BD Perm/Wash™ Buffer (Cat. No 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the USAGE section on the following page.

Usage

1. Resuspend 1 × 10^6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1× BD Perm/Wash™ Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (at RT or 4°C).
3. Wash twice in 100 µl of 1× BD Perm/Wash™ Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554603	Recombinant Human IL-2	10 µg	(none)
554563	Purified Rat Anti-Human IL-2	0.1 mg	MQ1-17H12
559317	PE Rat IgG2a κ Isotype Control	100 tests	R35-95
554723	Perm/Wash Buffer	100 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555334	PE-Cy™5 Mouse Anti-Human CD3	100 tests	UCHT1

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. This product is manufactured and sold under license from Pestka Biomedical Laboratories, Inc. (d/b/a PBL InterferonSource) and may be used solely as indicated. This product may not be resold or incorporated in any manner into another product for resale. Any use for therapeutics is strictly prohibited. This product is covered by U.S. Patent No. 5,597,901 and Bulgarian Patent No. BG1895.
7. Cy is a trademark of Amersham Biosciences Limited.
8. All other brands are trademarks of their respective owners.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1995:6.20-6.21. (Biology)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Biology)

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