Technical Data Sheet

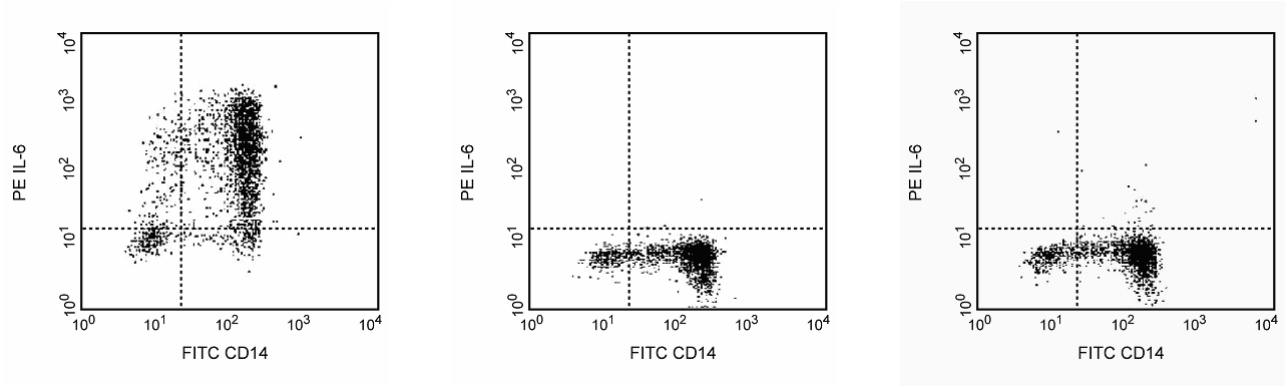
PE Rat Anti-Human IL-6

Product Information

Material Number:	**559331**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	MQ2-6A3
Immunogen:	Recombinant human IL-6
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MQ2-6A3 antibody reacts with human interleukin-6 (IL-6). The immunogen used to generate the MQ2-6A3 hybridoma was recombinant human IL-6. This is a neutralizing antibody.



Expression of IL-6 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 6 hours with LPS (100 ng/ml final concentration) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC-mouse anti-human CD14 monoclonal antibody (FITC-M5E2, Cat. No. 555397), fixed, permeabilized, and subsequently stained with 20 µl of PE-rat anti-human IL-6 antibody (PE-MQ2-6A3, Cat. No. 559331), by following the Usage section below and the BD Pharmingen™ staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scatter. To demonstrate specificity of staining, the binding by PE-MQ2-6A3 was blocked by each of the following: 1) preincubation of the conjugated antibody with recombinant human IL-6 (0.5 µg, Cat. No. 550071; middle panel) and by 2) preincubation of the fixed/permeabilized cells with unlabeled MQ2-6A3 antibody (2.5 µg; Cat. No. 559068; right panel) prior to staining with the PE-MQ2-6A3. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabeled antibody blocking specificity controls.*

Preparation and Storage

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.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The MQ2-6A3 antibody is useful for immunoflourescent staining and flow cytometric analysis to identify and enumerate human IL-6 producing cells within mixed cell populations. This 100 Test Size formulation of the PE-conjugated MQ2-6A3 antibody has been pre-titrated to assure effective intracellular detection of human IL-6 using 20 µl/1 x 10e6 cells.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MQ2-6A3 antibody with a ligand (e.g., recombinant human IL-6; Cat. No. 550071) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled MQ2-6A3 antibody (Cat. No. 559068) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe.

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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).

Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the USAGE section below.

USAGE

1. Resuspend 1 x 10e6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X 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 1X Perm/Wash™ Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
559317	PE Rat IgG2a κ Isotype Control	100 tests	R35-95
554723	Perm/Wash Buffer	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555063	Hick-3 Cytokine Positive Control Cells	1.0 ml	(none)
550071	Recombinant Human IL-6	10 µg	(none)
559068	Purified Rat Anti-Human IL-6	0.25 mg	MQ2-6A3

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 X 10e6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Andersson J, Abrams J, Bjork L, et al. Concomitant in vivo production of 19 different cytokines in human tonsils. *Immunology.* 1994; 83(1):16-24.(Clone-specific)

Andersson U, Andersson J. Immunolabeling of cytokine-producing cells in tissues and in suspension. In: Fradelizie D, Emelie D, ed. *Cytokine Producing Cells.* Paris: Inserm; 1994:32-49.(Clone-specific)

Litton M, Andersson J, Bjork L, Fehniger T, Ulfgren AK, Andersson U. Cytoplasmic cytokine staining in individual cells. In: Debets and Savelkoul, ed. *Human Cytokine Protocols.* Humana Press; 1996.(Clone-specific)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128.(Methodology: IC/FCM Block)