Technical Data Sheet

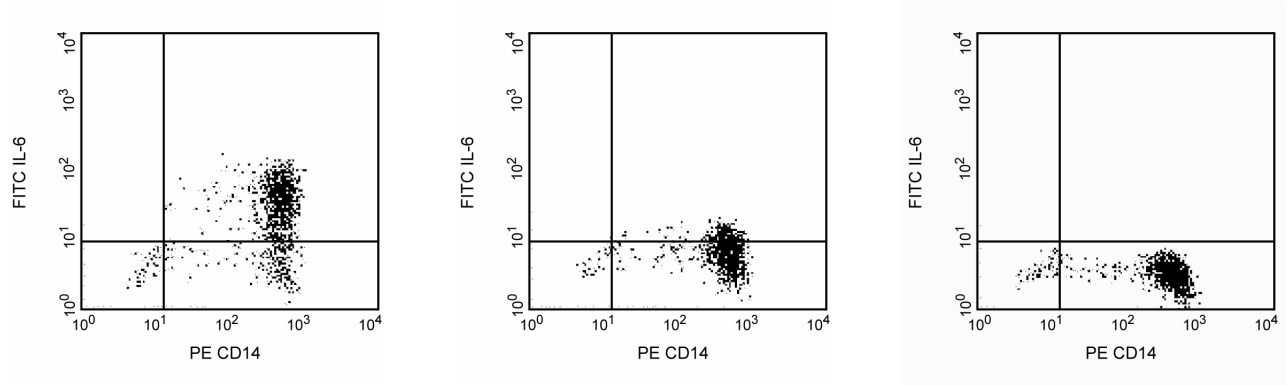
FITC Rat Anti-Human IL-6

Product Information

Material Number:	**554696**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	MQ2-6A3
Immunogen:	Recombinant human IL-6
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer 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.



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 BD GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with PE-mouse anti-human CD14 monoclonal antibody (PE-M5E2, Cat. No. 555398), fixed, permeabilized, and subsequently stained with 0.25 µg of FITC- rat anti-human IL-6 antibody (FITC-MQ2- 6A3, Cat. No. 554696), following BD Pharmingen's staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, the binding by the FITC-MQ2-6A3 antibody was blocked by preincubation of the conjugated antibody with recombinant human IL-6 protein (0.5 µg, Cat. No. 550071; middle panel) and by preincubation of the fixed/permeabilized cells with unlabeled MQ2-6A3 antibody (5.0 µg; Cat. No. 559068, right panel) prior to staining with the FITC-MQ2-6A3 antibody. 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 control.*

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 FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The MQ2-6A3 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate human IL-6 producing cells within mixed cell populations. FITC-conjugated MQ2-6A3 antibody (Cat. No. 554696) is especially suitable for these experiments (see figure). For optimal immunofluorescent staining for flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). A useful control for demonstrating specificity of staining is either of the following: 1) preblock the FITC- MQ2-6A3 antibody with 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. A suitable rat IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is FITC-R35-95 (Cat. No. 554688).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554688	FITC Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

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550071	Recombinant Human IL-6	10 µg	(none)
559068	Purified Rat Anti-Human IL-6	0.25 mg	MQ2-6A3
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555063	HiCK-3 Human Cytokine Positive Control Cells	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Use of these products to measure activation antigens expressed on mononuclear cell subsets for the purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US Patent Nos. 5,445,939, 5,656,446, 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australian Patent No. 615,880; and Japanese Patent No. 2,769,156.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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. (Biology)

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. (Biology)

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. (Biology)

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)

Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93. (Biology)

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