Technical Data Sheet

Purified Rat Anti-Human IL-6

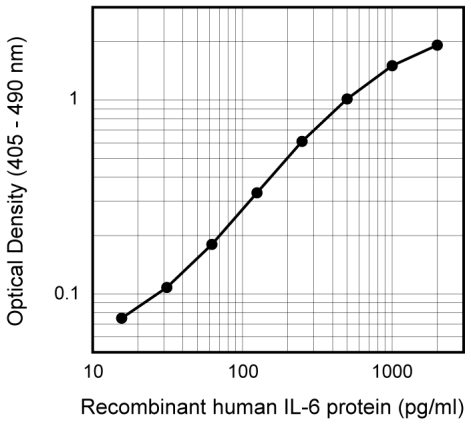
Product Information

Material Number:	**554543**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	MQ2-13A5
Isotype:	Rat IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MQ2-13A5 antibody reacts with human interleukin-6 (IL-6). The immunogen used to generate this hybridoma was COS-7 -expressed recombinant human IL-6. This is a neutralizing antibody.

This antibody is routinely tested by ELISA. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



ELISA standard curve for human IL-6.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

ELISA Capture	Routinely Tested
Intracellular block/flow cytometry	Tested During Development
Neutralization	Tested During Development
Western blot	Reported

Recommended Assay Procedure:

ELISA Capture: The purified MQ2-13A5 antibody (Cat. No. 554543) is useful as a capture antibody for a sandwich ELISA for measuring human IL-6 protein levels. Purified MQ2-13A5 antibody can be paired with biotinylated MQ2-Human IL-6 39C3 antibody (Cat. No. 554546) as the detecting antibody, with recombinant human IL-6 (Cat. No. 550071) as the standard. Purified MQ2-13A5 antibody should be titrated 1-4 µg/ml to determine optimal concentration for ELISA capture. To obtain linear standard curves, doubling dilutions of human IL-6 ranging from ~2,000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For specific methodology, please visit the protocols section or chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

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Note 1: This ELISA pair shows no cross-reactivity with any of the cytokines tested (e.g., mouse IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 p70, IL-15, GM-CSF, IFN-γ, MCP-1, TCA-3, TNF; human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, G-CSF, GM-CSF, IFN-γ, lymphotactin, MCP-1, MCP-2, MIP-1α, MIP-1β, NT-3, PDGF-AA, sCD23 , SCF, TNF, LT-β, VEGF; rat IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, TNF).

Note 2: This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. These ELISA reagents are not recommended for assay of serum samples. The BD OptEIA™ ELISA Set (Cat. No. 555220) and Kit (Cat. No. 550799) are specially-formulated for serum cytokine measurement.

Other Applications

1. Blocking Control for Intracellular Staining: The purified MQ2-13A5 antibody can be used as a blocking control to demonstrate specificity of IL-6 staining by the FITC-MQ2-13A5 antibody (Cat. No. 554544) or the PE-MQ2-13A5 antibody (Cat. No. 554545). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1-10 µg of purified MQ2-13A5 antibody (Cat. No. 554543) for 20 minutes at 4°C, prior to staining with the conjugated antibody (e.g., 0.1 - 0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

2. IF/Flow: The MQ2-13A5 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-6 producing cells within mixed cell populations. The directly- conjugated MQ2-13A5 antibodies are especially suitable for these studies. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

3. Western Blot: The MQ2-13A5 antibody (Cat. No. 554543) has been found useful for Western blot. Please note that this application is not routinely tested at BD Biosciences.

3. Neutralization: The NA/LE formulation of the MQ2-13A5 antibody (Cat. No. 554541) is useful for neutralization of human IL-6 bioactivity.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554546	Biotin Rat Anti-Human IL-6	0.5 mg	MQ2-39C3
550071	Recombinant Human IL-6	10 µg	(none)
555220	Human IL-6 ELISA Set	20 tests	(none)
550799	Human IL-6 ELISA Kit II	2 plates	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.

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.(Clone-specific: ELISA, Neutralization)

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.(Clone-specific: ELISA, Neutralization)

Gaines Das RE, Poole S. The international standard for interleukin-6. Evaluation in an international collaborative study. *J Immunol Methods.* 1993; 160(2):147-153. (Clone-specific: ELISA, Neutralization)

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)