Technical Data Sheet

Purified Rat Anti-Mouse IL-6

Product Information

Material Number:	**554400**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	MP5-20F3
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP5-20F3 antibody reacts with mouse interleukin-6 (IL-6). The immunogen used to generate the MP5-20F3 hybridoma was recombinant mouse IL-6. This is a neutralizing antibody.

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 MP5-20F3 antibody (Cat. No. 554400) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-6 protein levels. Purified MP5-20F3 antibody can be paired with the biotinylated MP5-32C11 antibody (Cat. No. 554402) as the detecting antibody, with recombinant mouse IL-6 (Cat. No. 554582) as the standard. Purified MP5-20F3 antibody should be titrated 2-6 µg/ml to determine optimal concentration for ELISA capture. To obtain linear standard curves, doubling dilutions of mouse IL-6 ranging from ~2000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For specific methodology please visit the protocols sections or the chapter on ELISA in the Immune Function Handbook, both of which are posted on our website, www.bdbiosciences.com.

Note: This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. These ELISA reagents are not recommended for assaying serum or plasma samples. For measuring mouse IL-6 in serum or plasma our mouse IL-6 BD OptEIA™ set (Cat. No. 555240) or BD OptEIA™ Kit (Cat. No. 550950) are specifically formulated and recommended.

Blocking Control for Intracellular Staining: The purified MP5-20F3 antibody (Cat. No. 554400) can be used as a blocking control to demonstrate specificity of IL-6 staining by the PE-conjugated MP5-20F3 antibody (Cat. No. 554401). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1 - 10 µg of purified MP5-20F3 antibody (Cat. No. 554400) for 20 minutes at 4°C, prior to staining with the PE-MP5-20F3 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. 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 website, www.bdbiosciences.com.

Neutralization: The BD NA/LE™ MP5-20F3 antibody (Cat. No. 554398) is useful for neutralization of mouse IL-6 bioactivity. A suitable BD NA/LE™ rat IgG1 isotype-matched control is the R3-34 antibody (Cat. No. 554682).

Western Blot: The MP5-20F3 antibody has been reported to be useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554582	Recombinant Mouse IL-6	5 µg	(none)
554402	Biotin Rat Anti-Mouse IL-6	0.5 mg	MP5-32C11
555240	Mouse IL-6 ELISA Set	20 tests	(none)
550950	Mouse IL-6 ELISA Kit	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)

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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)

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Starnes HF Jr, Pearce MK, Tewari A, Yim JH, Zou JC, Abrams JS. Anti-IL-6 monoclonal antibodies protect against lethal Escherichia coli infection and lethal tumor necrosis factor-alpha challenge in mice. *J Immunol.* 1990; 145(12):4185-4191.(Clone-specific: Neutralization)

Suda T, O'Garra A, MacNeil I, Fischer M, Bond MW, Zlotnik A. Identification of a novel thymocyte growth-promoting factor derived from B cell lymphomas. *Cell Immunol.* 1990; 129(1):228-240.(Clone-specific: Neutralization)