Technical Data Sheet

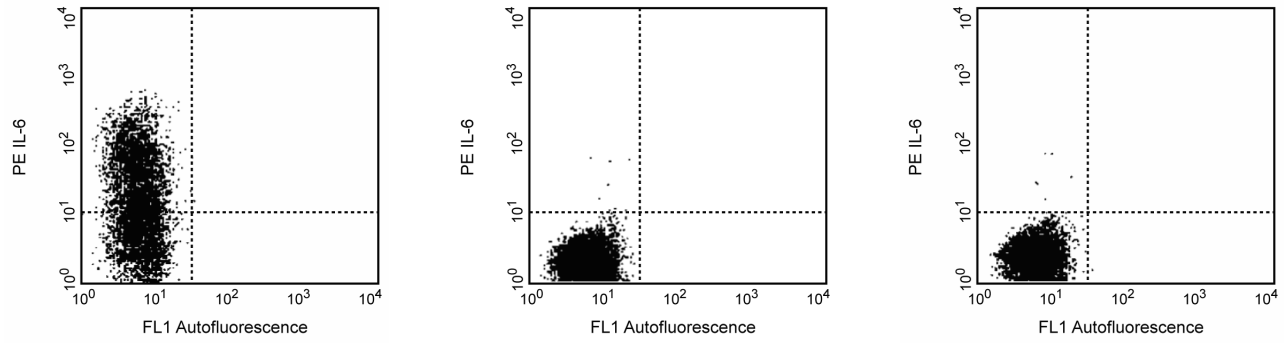
PE Rat Anti-Mouse IL-6

Product Information

Material Number:	**562050**
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	MP5-20F3
Immunogen:	Mouse IL-6 Recombinant Protein
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP5-20F3 monoclonal antibody specifically binds to mouse interleukin-6 (IL-6). The immunogen used to generate the MP5-20F3 hybridoma was recombinant mouse IL-6.



Expression of IL-6 by stimulated BALB/c cells. *BALB/c bone marrow cells were cultured for 2 weeks in the presence of recombinant mouse GM-CSF (20 ng/ml; Cat. No. 554586). The cells were washed and stimulated with recombinant mouse IFN-γ (10 ng/ml; Cat. No. 554587) for 4 hours followed by an overnight stimulation with LPS (1 µg/ml; Sigma, Cat. #L-8272) and BD GolgiPlug™ (1 µg/ml; Cat. No. 555029; aka Brefeldin A). Adherent cells were incubated with 1x trypsin EDTA at 37°C for 10 minutes and gently dislodged by pipetting for cell harvest. Nonspecific binding of cell surface antigens was blocked by incubation of the cells withMoouse BD Fc Block™ (10 µg/ml; Cat. No. 553142). The cells were fixed , permeabilized then subsequently stained with 0.06 µg of PE Rat anti-Mouse IL-6 antibody by using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-MP5-20F3 antibody was blocked by preincubation with recombinant mouse IL-6 (0.12 µg; Cat. No. 554582; middle panel) or by preincubation of the fixed/permeabilized cells with unlabeled MP5-20F3 antibody (5 µg; Cat. No. 554400; right panel) prior to staining. 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

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 MP5-20F3 antibody can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate IL-6-producing cells within mixed cell populations (see images). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.25 µg mAb/million cells) For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Other applications for this clone in additional formats available are:

Neutralization: The NA/LE™ MP5-20F3 antibody (Cat. No. 554398) is useful for neutralization of mouse IL-6 bioactivity.

ELISA Capture: The purified MP5-20F3 antibody (Cat. No. 554401) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-6 protein levels with the biotinylated detection antibody (Cat. No. 554402) and recombinant mouse IL-6 (Cat. No. 554582) as a standard.

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554586	Recombinant Mouse GM-CSF	10 µg	(none)
554587	Recombinant Mouse IFN-γ	10 µg	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554582	Recombinant Mouse IL-6	5 µg	(none)
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554400	Purified Rat Anti-Mouse IL-6	0.5 mg	MP5-20F3
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554654	MiCK-3 Mouse 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome 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. An isotype control should be used at the same concentration as the antibody of interest.

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)

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)

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: Flow cytometry, IC/FCM Block)

Sander B, Hoiden I, Andersson U, Moller E, Abrams JS. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. Cytokine detection by immunoassay and intracellular immunostaining. *J Immunol Methods.* 1993; 166(2):201-214. (Clone-specific: ELISA, Flow cytometry)

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)