Technical Data Sheet

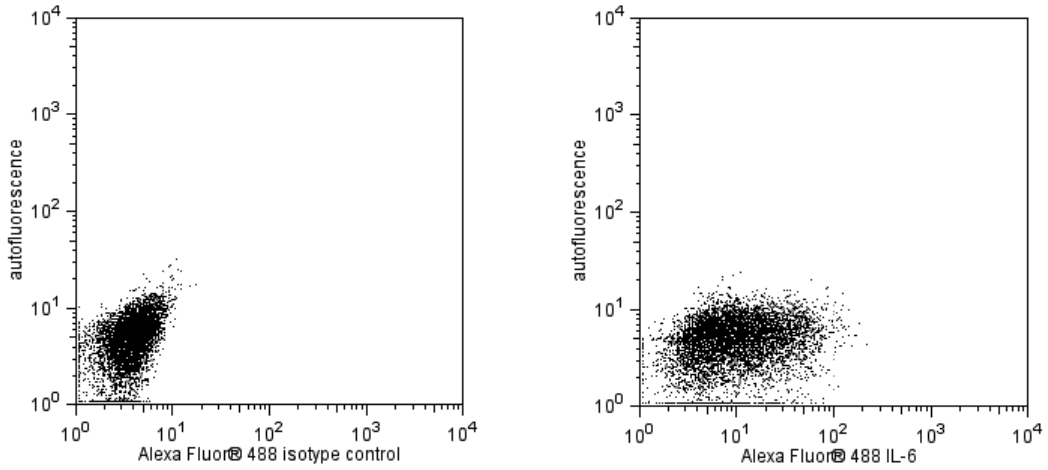
Alexa Fluor® 488 Rat Anti-Mouse IL-6

Product Information

Material Number:	**561363**
Alternate Name:	Il6; Il-6; Interleukin-6; B-cell hybridoma growth factor
Size:	50 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.



Flow cytometric analysis of intracellular IL-6 expression by activated mouse macrophages. *Thioglycollate-elicited mouse peritoneal macrophages were primed with recombinant mouse IFN-γ (10 ng/ml, Cat. No. 554587) for 2 hr and stimulated overnight with lipopolysaccharide (LPS, Sigma, Cat. No. L-8272; 1 µg/ml) and BD GolgiPlug™ Protein Transport Inhibitor (Containing Brefeldin A) (Cat. No. 555029). The adherent cells were washed with 1× phosphate buffered saline (PBS) and incubated with 1× trypsin-EDTA solution (37°C, 15 min). The cells were harvested, washed, incubated with Fc Block™ (Rat IgG2b,κ Anti-Mouse CD16/CD32) antibody (Cat. No. 553142), fixed and permeabilized using BD Cytofix™ Fixation Buffer (Cat. No. 554655) and BD Perm/Wash™ Buffer (Cat. No. 554723). The cells were then stained either with an Alexa Fluor® 488 Rat IgG1, κ Isotype Control (Cat No. 557720, Left Panel) or with the Alexa Fluor® 488 Rat Anti-Mouse IL-6 antibody (Cat No. 561363, Right Panel). MiCK-3 Mouse Cytokine Positive Control Cells (Cat No. 554654) are prepared in a similar manner. These cells can be used as a positive control for cytokine flow cytometry experiments designed to characterize the nature of mouse IL-6-producing cells. Two-color flow cytometric dot plots showing the correlated expression of IL-6 (or Ig Isotype control staining) versus cellular autofluorescence measured in the phycoerythrin channel (autofluorescence) were derived from events with the forward and side light-scatter characteristics of intact macrophages. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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 to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

BD Biosciences

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
557720	Alexa Fluor® 488 Rat IgG1 κ Isotype Control	0.1 mg	R3-34
554656	Stain Buffer (FBS)	500 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554654	MiCK-3 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554587	Recombinant Mouse IFN-γ	10 µg	(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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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. (Biology)

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. (Biology: 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. (Biology: 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. (Biology: 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. (Biology: Neutralization)