Technical Data Sheet

V450 Rat Anti-Mouse IL-6

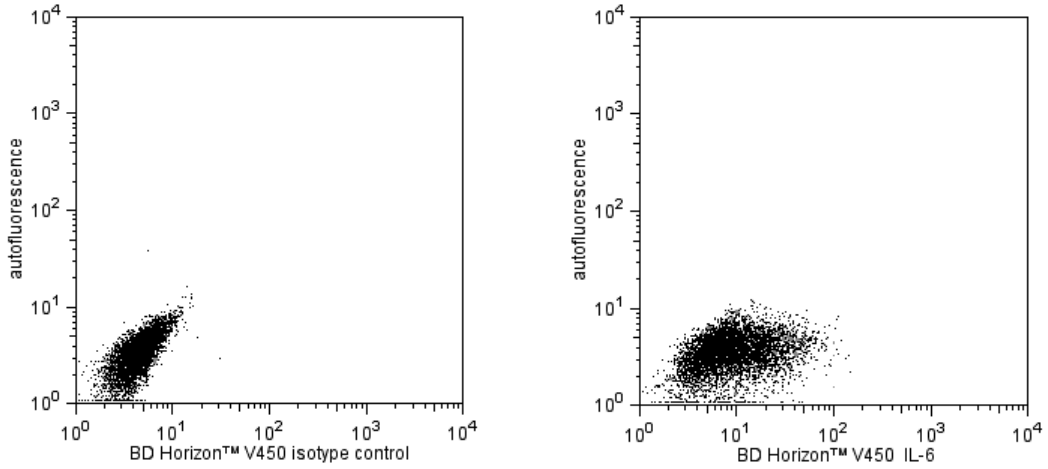
Product Information

Material Number:	**561376**
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 protein stabilizer and ≤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.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



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 a BD Horizon™ V450 Rat IgG1, κ Isotype Control (Cat No. 560535, Left Panel) or with the BD Horizon™ V450 Rat Anti-Mouse IL-6 antibody (Cat No. 561376, 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.*

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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 BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

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)
560535	V450 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554587	Recombinant Mouse IFN-γ	10 µg	(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)

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. 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.
4. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
5. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. 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)

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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. (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)