Technical Data Sheet

V450 Rat Anti-Mouse IL-10

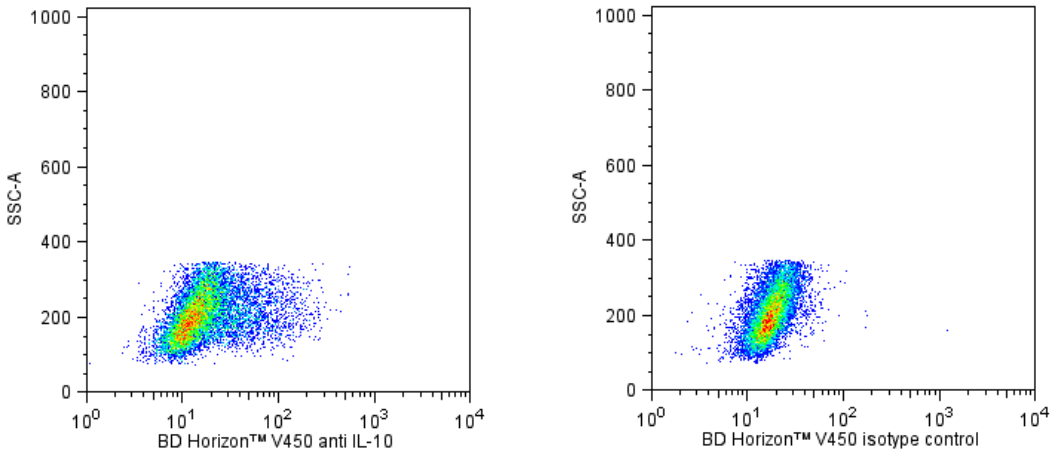
Product Information

Material Number:	**561429**
Alternate Name:	Interleukin-10; Il10; CSIF; Cytokine synthesis inhibitory factor
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	JES5-16E3
Immunogen:	Recombinant mouse IL-10
Isotype:	Rat IgG2b
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The JES5-16E3 monoclonal antibody specifically binds to the mouse cytokine, Interleukin-10 (IL-10). IL-10 is also known as Cytokine Synthesis Inhibitory Factor (CSIF). It is produced by various activated cell types including CD4+ T cells, CD8+ T cells, NK T cells, B1 B cells, NK cells, macrophages, dendritic cells, mast cells, granulocytes and keratinocytes. IL-10 plays a pivotal role in regulating immune responses and protecting the host from damage caused by inflammatory and autoimmune responses. IL-10 has numerous biological activities including the inhibition of cytokine synthesis by activated T cells, NK cells, monocytes, and macrophages. In the presence of accessory cells, IL-10 inhibits mitogen- or anti-CD3 induced proliferation of T lymphocytes. IL-10 has also been shown to costimulate the development of thymocytes, B cell differentiation and the generation of cytotoxic T cells. The immunogen used to generate the JES5-16E3 hybridoma was recombinant mouse IL-10. JES5-16E3 is a neutralizing antibody.

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.



Expression of intracellular IL-10 by stimulated mouse splenic T lymphocytes. *An enriched population of mouse splenic CD4+ T cells was stimulated for 2 days with immobilized anti-Mouse CD3 and anti-Mouse CD28 antibodies in culture along with recombinant mouse IL-2 and IL-4. The stimulated cells were harvested and cultured for 3 days in tissue culture medium supplemented with recombinant IL-2 and IL-4. The cells were then collected and stimulated with Leukocyte Activation Cocktail with BD GolgiPlug™ (Cat. No. 550583) that contains Phorbol 12-Myristate 13-Acetate (PMA), ionomycin and a protein transport inhibitor, Brefeldin A. After 4 hours, the cells were harvested, washed, and fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655). After washing, the cells were permeabilized with Perm/Wash Buffer (Cat No. 554723) and stained with BD Horizon™ V450 Rat anti-Mouse IL-10 antibody (Cat No. 561429, Left Panel) or BD Horizon™ V450 Rat IgG2b Isotype Control (Cat No. 560457, Right Panel). Flow cytometric dot plots showing correlated expression of IL-10 (or Ig isotype control staining) versus side scattered-light signals were derived from events with the forward and side light-scatter characteristics of lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

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.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
560457	V450 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
550583	Leukocyte Activation Cocktail, with BD GolgiPlug™	200 µl	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
550069	Recombinant Mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
554653	MiCK-2 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. 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.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.

References

Andersson U, Andersson J. Immunolabeling of cytokine-producing cells in tissues and in suspension. In: Fradelizie D, Emelie D, ed. *Cytokine Producing Cells.* Paris: Inserm; 1994:32-49. (Clone-specific: Immunocytochemistry (cytospins), Neutralization)

Litton MJ, Sander B, Murphy E, O'Garra A, Abrams JS. Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B. *J Immunol Methods.* 1994; 175(1):47-58. (Clone-specific: Neutralization)

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: Immunocytochemistry (cytospins), Neutralization)

Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol.* 2010; 10(3):170-181. (Biology)