Technical Data Sheet

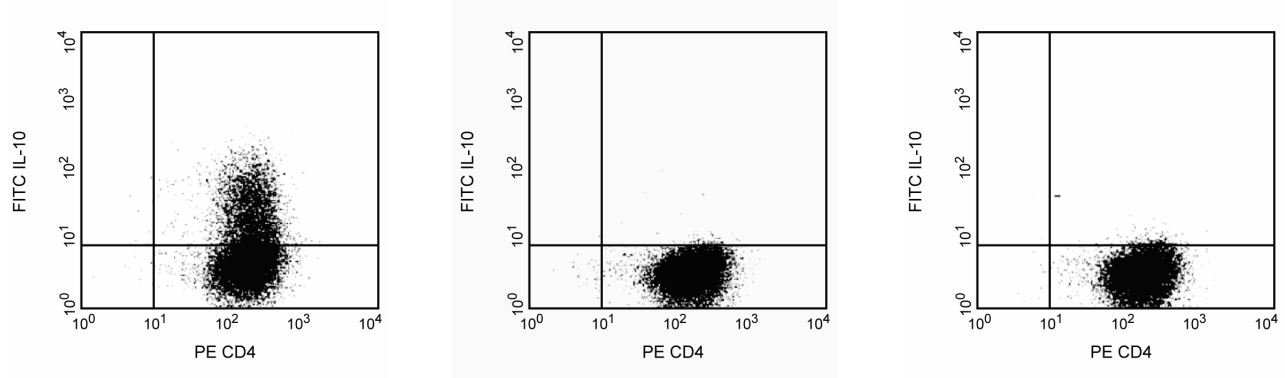
FITC Rat Anti-Mouse IL-10

Product Information

Material Number:	**562037**
Size:	25 µg
Concentration:	0.5 mg/ml
Clone:	JES5-16E3
Immunogen:	Recombinant mouse IL-10
Isotype:	Rat IgG2b
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤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.



Expression of IL-10 by stimulated CD4+ Balb/c spleen cells. *Purified splenic CD4+ cells from 6 month old BALB/c mice were stimulated with plate-bound anti-CD3 (145-2C11, Cat. No. 553057 at 25 µg/ml) and soluble anti-mouse CD28 (clone 37.51, Cat. No. 553294 at 2 µg/ml) for 2 days in culture together with recombinant mouse IL-2 (10 ng/ml, Cat. No. 550069) and recombinant mouse IL-4 (1 ng/ml, Cat. No. 550067), followed by a 3 day incubation with only recombinant IL-2 and IL-4. This was followed by a 5 hour stimulation with plate-bound anti-CD3 (25 µg/ml) and anti-mouse CD28 (2 µg/ml) in the presence of 2 µM monensin (BD GolgiStop™ Cat. No. 554724). The cells were harvested, stained with PE Rat anti-Mouse CD4 (RM4-5, Cat. No. 553049), fixed, permeabilized, and subsequently stained with FITC Rat anti-Mouse IL-10 (JES5-16E3, Cat. No. 562037) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining, the binding of FITC-JES5-16E3 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse IL-10 (Cat. No. 550070; middle panel), and by preincubation of the fixed/permeabilized cells with an excess of the unlabelled JES5-16E3 antibody (Cat. No. 554464; right panel). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) and unlabelled antibody blocking (right panel) specificity controls. A suitable rat IgG2b isotype control for assessing background staining on fixed/permeabilized mouse cells is FITC-A95-1 (Cat. No. 556923); use at comparable concentrations to antibody of interest.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC 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

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Recommended Assay Procedure:

Immunofluorescent Staining for Flow Cytometric Analysis: The JES5-16E3 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-10 producing cells within mixes cell populations. The FITC-conjugated JES516E3 antibody (Cat. No. 554466;) is especially suitable for these experiments (see figure, left panel). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/ million cells). The use of a specificity control, such as one of the following, is suggested: 1) recombinant mouse IL-10 (Cat. No. 550070) unlabeled JES5-16E3 antibody, (Cat. No. 554464), and 3) rat IgG2b isotype control, FITC-A95-1 (Cat. No. 556923). Isotype control should be used at comparable concentration to antibody of interest. For specific methodology, visit the protocols section of the website, or see the *Techniques for Immune Function Analysis Application Handbook, Chapter 4: Immunofluorescent Staining of Intracellular Molecules for Flow Cytometric Analysis*, both of which are located at www.bdbiosciences.com.

Neutralization: The NA/LE™ FES5-16E3 antibody (Cat. No. 554463) is useful for neutralization of mouse IL-10 bioactivity.

ELISA Detection: The biotinylated JES5-16E3 antibody (Cat. No. 554465) is useful as a detection antibody for a sandwich ELISA for measuring mouse IL-10 protein levels. For testing IL-10 in serum or plasma, our OptEIA™ Set (Cat. No. 555252) is recommended.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
556923	FITC Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
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
550069	Recombinant Mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

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)

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)

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)