Technical Data Sheet

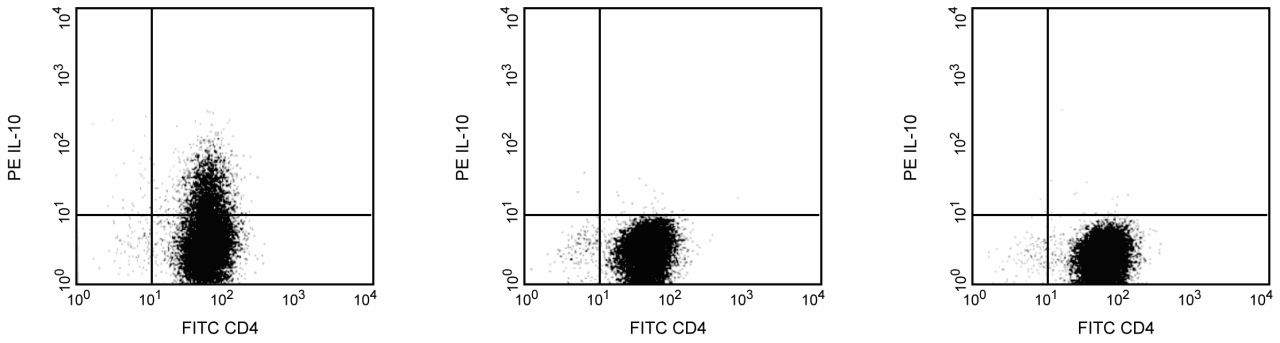
PE Rat Anti-Mouse IL-10

Product Information

Material Number:	**554467**
Size:	0.1 mg
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 ≤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 (clone 145-2C11, Cat. No. 553058 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 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 GolgiStop™ (Cat. No. 554724). The cells were then stained with 0.06 µg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553046) and 0.12 µg of PE-conjugated rat anti-mouse IL-10 antibody (PE-JES5-16E3, Cat. No. 554467) by using BD Pharmingen's staining protocol. To demonstrate specificity of staining, the binding of PE-JES5-16E3 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse IL-10 (0.12 µg, Cat. No.550070; middle panel), and by preincubation of the fixed and permeabilized cells with an excess of the unlabelled JES5-16E3 mAb (3.6 µg, 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 levels of background staining on fixed/permeabilized mouse cells is PE-R35-38 (Cat. No. 556925); 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 R-PE under optimum conditions, and unconjugated antibody and free PE were 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

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877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

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Recommended Assay Procedure:

The PE-conjugated JES5-16E3 antibody can be used for multicolor flow cytometric analyses to identify and enumerate IL-10 producing cells within mixed cell populations (see figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, visit our web site, www.bdbiosciences.com, and go to the protocols section or the Techniques for Immune Function Analysis Application Handbook, Chapter 4: Immunofluorescent Staining of Intracellular Molecules for Flow Cytometric Analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553058	Purified 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)
556925	PE Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554653	MiCK-2 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.

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)