

Technical Data Sheet

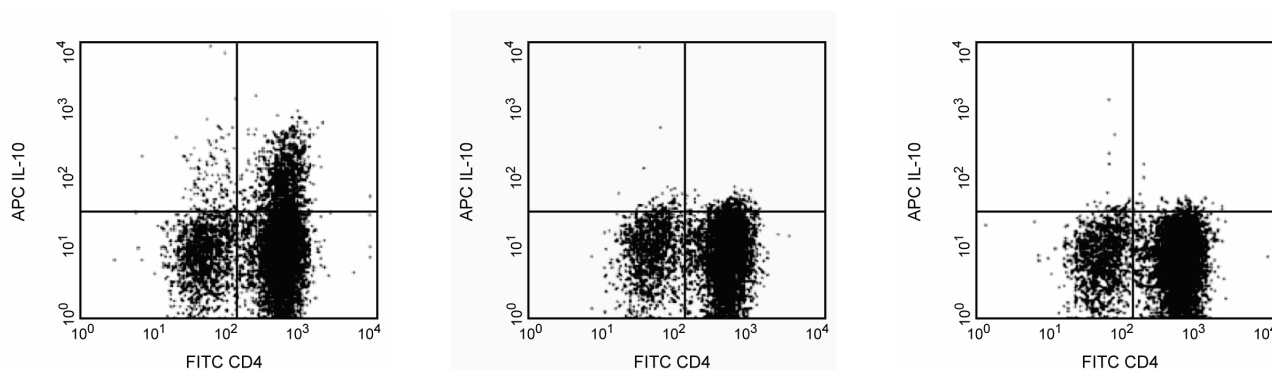
APC Rat Anti-Mouse IL-10

Product Information

Material Number:	554468
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 (145-2C11, Cat. No. 553057 at 25 µg/ml) and soluble anti-mouse CD28 (clone 37.51, Cat. No. 553296 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 (50 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 0.25 µg of APC-conjugated rat anti-mouse IL-10 antibody (APC-JES5-16E3, Cat. No. 554468) by using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of APC-JES5-16E3 was blocked by the preincubation of the conjugated antibody with a molar excess of recombinant mouse IL-10 (0.12 µg, Cat. No. 550070; middle panel), and by preincubation of the fixed/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 unlabeled antibody blocking (right panel) specificity controls. A suitable rat IgG2b isotype control for assessing levels of background staining on fixed/permeabilized mouse cells is APC-R35-38 (Cat. No. 556924); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg / 1 million cells). This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.

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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 to APC under optimum conditions, and unconjugated antibody and free APC 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
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Recommended Assay Procedure:

Immunofluorescent Staining for Flow Cytometric Analysis: The APC-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 image). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated ($\leq 0.5 \mu\text{g}$ mAb/million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or Chapter 4: Immunofluorescent Staining of Intracellular Molecules for Flow Cytometric Analysis in the *Techniques for Immune Function Analysis Application Handbook 1st Edition. 2003*.

ELISA: The purified JES5-16E3 is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-10 protein levels.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553296	Biotin 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)
556924	APC Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(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. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
5. 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)