Technical Data Sheet

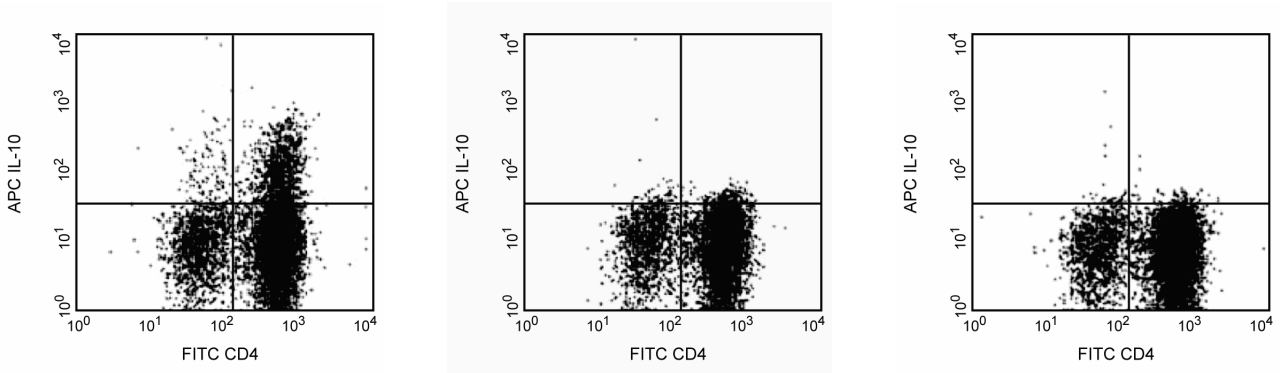
APC Rat Anti-Mouse IL-10

Product Information

Material Number:	**561059**
Size:	25 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 ≤0.09% sodium azide.

Description

The JES5-16E3 antibody reacts with mouse interleukin-10 (IL-10). The immunogen used to generate the JES5-16E3 hybridoma was recombinant mouse IL-10.



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 (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) (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 JES516E3 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 a dye, HeNE or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.*

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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry: 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. For optimal immunofluorescent staining with flow cytometric analysis, this antibody should be titrated (≤ 0.5 µg mAb/million cells).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
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)
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
550070	Recombinant Mouse IL-10	10 µg	(none)
554464	Purified Rat Anti-Mouse IL-10	0.1 mg	JES5-16E3

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
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.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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. (Biology: 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. (Biology: 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)

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. (Biology: Immunocytochemistry (cytospins), Neutralization)