Technical Data Sheet

Purified Rat Anti-Mouse IL-10

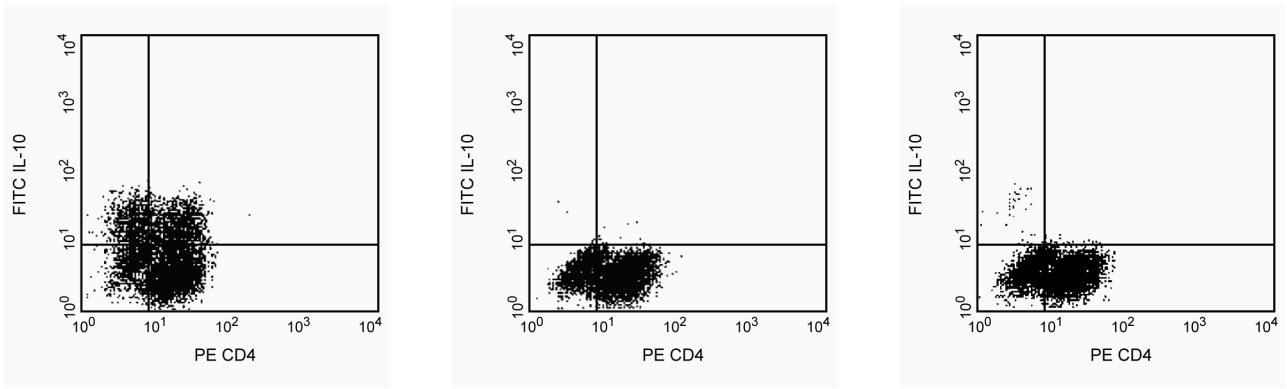
Product Information

Material Number:	**554464**
Size:	0.1 mg
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 antibody reacts with mouse interleukin-10 (IL-10). The immunogen used to generate the JES5-16E3 hybridoma was recombinant mouse IL-10. This is a neutralizing antibody.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



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 (25 µg/ml final concentration; 145-2C11, Cat. No. 553057) and soluble anti-mouse CD28 (2 µg/ml final concentration; clone 37.51, Cat. No. 553294) for 2 days in culture together with recombinant mouse IL-2 (10 ng/ml final concentration; Cat. No. 550069) and recombinant mouse IL-4 (50 ng/ml final concentration; Cat. No.550067), followed by a 3 day incubation with only recombinant mouse IL-2 and recombinant mouse IL-4. This was followed by a 4 hour stimulation with PMA (5 ng/ml final concentration) and ionomycin (500 ng/ml final concentration) in the presence of GolgiPlug™ (1 µg/ml final concentration; Cat. No. 555029). The cells were then stained with 0.06 µg of PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553050) and subsequently fixed, permeabilized, and stained with 0.12 µg of FITC-conjugated rat anti-mouse IL-10 antibody (FITC-JES5-16E3, Cat. No. 554466) by using Pharmingen's staining protocol. To demonstrate specificity of staining, the binding of the FITC-JES5-16E3 antibody was blocked by preincubation of the conjugated antibody with recombinant mouse IL-10 (0.25 µg; Cat. No. 550070; see middle panel), and by preincubation of the fixed/permeabilized cells with of the unlabeled JES5-16E3 mouse antibody (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 PE-R35-38 (Cat. No. 555848); 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.
Store undiluted at 4° C.

Application Notes

Application

Intracellular block/flow cytometry	Tested During Development

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Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified JES5-16E3 antibody (Cat. No. 554464) can be used as a blocking control to demonstrate specificity of IL-10, staining by FITC-JES5-16E3, PE-JES5-16E3 and APC-JES5-16E3 antibody (Cat. No. 554466; 554467; 554468). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1 - 10 µg of unlabeled JES5-16E3 antibody (Cat. No. 554464) for 20 minutes at 4°C, prior to staining with FITC-JES5-16E3, PE-JES5-16E3 or APC-JES5-16E3 antibody (e.g., 0.1 - 0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Neutralization: The JES5-16E3 antibody neutralizes mouse IL-10 bioactivity. Our no azide/low endotoxin (NA/LE™) antibodies are formulated for in vivo and in vitro assays. The NA/LE™ JES5-16E3 antibody (Cat. No. 554463) is the recommended format for use in neutralization.

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, the Mouse IL-10 OptEIA™ Set, Cat. No. 555252 is recommended.

Immunofluorescent Staining and 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 mixed cell populations. The FITC-, PE-, and APC-conjugated JES5-16E3 antibodies (Cat. No. 554466; 554467; 554468) are especially suitable for these experiments.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
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)
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11

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.

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: IC/FCM Block)

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)