Technical Data Sheet

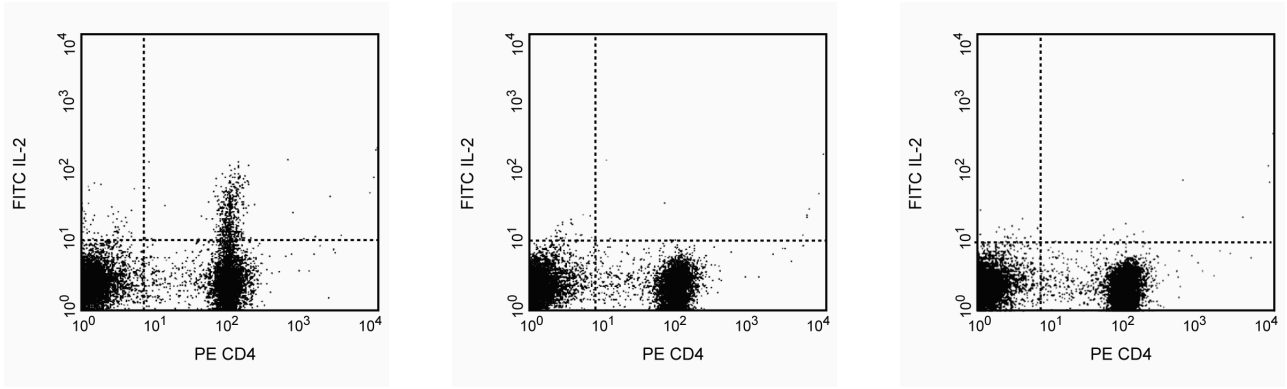
Purified Rat Anti-Mouse IL-2

Product Information

Material Number:	**554425**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	JES6-5H4
Immunogen:	Mouse IL-2 Recombinant Protein
Isotype:	Rat IgG2b
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The JES6-5H4 antibody reacts with mouse interleukin-2 (IL-2) and has been reported to be neutralizing.



Expression of IL-2 by stimulated CD4+ and CD4- BALB/c spleen cells. *Splenocytes from 6 month old BALB/c mice were stimulated for 5 hours with hamster anti-mouse CD3 (2 µg/ml final concentration; clone 145-2C11, Cat. No. 553057) and hamster anti-mouse CD28 (2 µg/ml final concentration; clone 37.51, Cat. No. 553294) antibodies in the presence of BD GolgiStop™ (3 µM final concentration; Cat. No. 554724). The cells were harvested, stained with 0.06 µg of PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553049), fixed, permeabilized, and subsequently stained with 0.06 µg of FITC-conjugated rat anti-mouse IL-2 antibody (FITC-JES6-5H4, Cat. No. 554427, left panel). To demonstrate specificity of staining, the binding by the FITC-JES6-5H4 antibody was blocked by preincubation of the conjugated antibody with recombinant mouse IL-2 (0.12 µg, Cat. No. 550069; middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled JES6-5H4 antibody (2.5 µg; Cat. No. 554425; right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabelled antibody blocking specificity controls.*

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	Routinely Tested
ELISA	Tested During Development
Immunoprecipitation	Reported

Recommended Assay Procedure:

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Blocking Control for Intracellular Staining: The purified JES6-5H4 antibody can be used as a blocking control to demonstrate specificity of IL-2 staining by directly conjugated-JES6-5H4 antibodies. To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1-10 µg of unlabeled JES6-5H4 antibody (Cat. No. 554425) for 20 minutes at 4°C, prior to staining with conjugated-JES6-5H4 (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. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

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ELISA Detection: The biotinylated JES6-5H4 antibody (Cat. No. 554426) is useful as a detection antibody for a sandwich ELISA for measuring mouse IL-2 protein levels.

IP: The purified JES6-5H4 antibody has been reported useful for immunoprecipitation. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
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
554427	FITC Rat Anti-Mouse IL-2	0.1 mg	JES6-5H4
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554652	MiCK-1 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. 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. Use of these products to measure activation antigens expressed on mononuclear cell subsets for the purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US Patent Nos. 5,445,939, 5,656,446, 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australian Patent No. 615,880; and Japanese Patent No. 2,769,156.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1995:6.20-6.21. (Clone-specific: ELISA)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific: ELISA, Immunoprecipitation)

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. (Clone-specific: ELISA)