Technical Data Sheet

FITC Rat Anti-Mouse IL-2

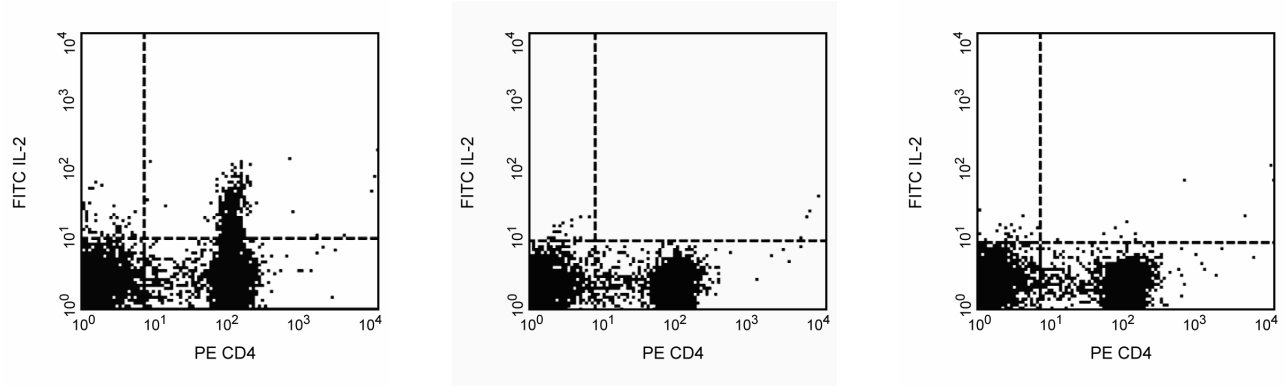
Product Information

Material Number:	**554427**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	JES6-5H4
Immunogen:	Recombinant mouse IL-2
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). The immunogen used to generate the JES6-5H4 hybridoma was recombinant mouse IL-2. 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-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 mg/ml final concentration; Cat. No.553057, clone 145-2C11) and hamster anti-mouse CD28 (2 mg/ml final concentration; Cat. No. 553294, clone 37.51) 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) using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding by FITC-JES6-5H4 was blocked by each of the following: 1) preincubation of the conjugated antibody with recombinant mouse IL-2 (0.12 mg, Cat. No. 550069; middle panel), and by 2) preincubation of the fixed/permeabilized cells with excess unlabelled JES6-5H4 antibody 2.5 mg; Cat. No. 554425; right panel) prior to staining with the FITC-JES6-5H4 antibody. 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. A suitable rat IgG2b isotype control for assessing the level of background staining on fixed/permeabilized mouse cells is Cat. No. 556923; 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 FITC under optimum conditions, and unreacted FITC was 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 and Flow Cytometric Analysis: The JES6-5H4 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-2 producing cells within mixed cell populations. FITC-, PE-, APC, Alexa Fluor® 488 and Alexa Fluor® 647 conjugated antibodies (Cat. No. 554427; Cat. No. 554428; Cat No. 554429, Cat. No. 557725 and Cat. No. 557736) are especially suitable for these studies.

An appropriate rat IgG2b isotype control is Cat. No. 556923. A useful control for demonstrating specificity of staining is pre-blocking with one of the following: 1) recombinant mouse IL-2 (Cat. No. 550069) or 2) unlabeled JES6-5H4 antibody (Cat. No. 554425), prior to staining. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554652	MiCK-1 Cytokine Positive Control Cells	NA	(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
556923	FITC Rat IgG2b, κ Isotype Control	0.1 mg	A95-1

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.
5. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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)