Technical Data Sheet

Alexa Fluor® 488 Rat Anti-Mouse IL-2

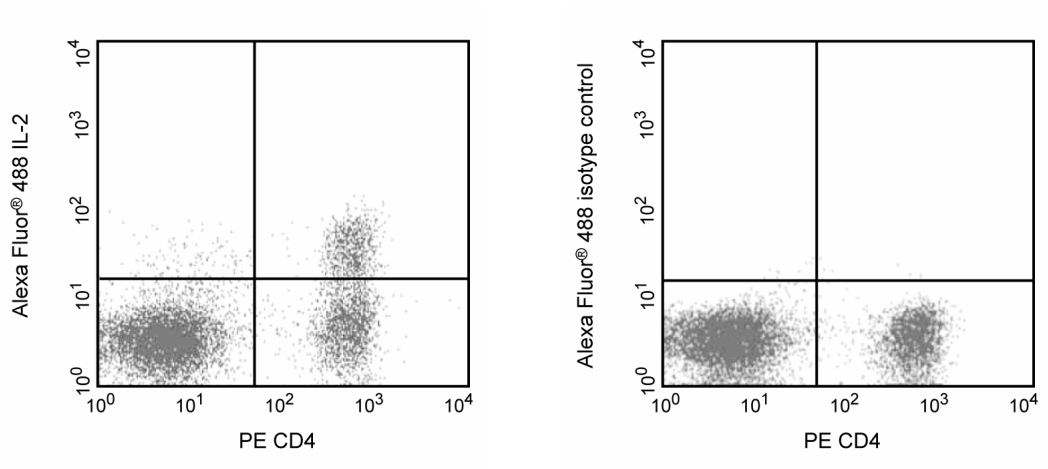
Product Information

Material Number:	**557725**
Size:	0.1 mg
Concentration:	0.2 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 BALB/c mice were stimulated for 4 hours with PMA (5 ng/ml, Sigma Cat. No. P-8139) and Ionomycin (500 ng, Sigma Cat. No. I-0634) in the presence of Brefeldin A (BD GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553048) and either rat anti-mouse IL-2 antibody (Alexa Fluor® 488-JES6-5H4, Cat. No. 557725), (left panel) or Rat IgG2b isotype control (Alexa Fluor® 488-A95-1, Cat. No. 557726) , (right panel) by using Pharmingen's staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 488 conjugate was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse IL-2 (0.25 µg, Cat. No. 550069, data not shown) prior to staining. Dot plots were derived from gated events with the forward and side light scatter characteristics of lymphocytes. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 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

Recommended Assay Procedure:

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Immunofluorescent Staining for 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. Alexa Fluor® 488-conjugated antibody (Cat. No. 557725) is especially suitable for these studies. For optimal immunofluorescent staining for flow cytometric analysis, the anti-cytokine

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antibody should be titrated. For specific methodology, please visit the protocols section or chapter on intracellular immunofluorescent staining in the Immune Function Handbook, both of which are posted on our website, www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the PE-conjugated JES6-5H4 antibody with ligand (e.g., recombinant mouse IL-2, Cat. No. 550069) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled JES6-5H4 antibody (Cat. No. 554425) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG2b isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse cells is Alexa Fluor® 488-A95-1 (Cat. No. 557726); use at comparable concentrations to antibody of interest. 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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557726	Alexa Fluor® 488 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	5x10^6 cells	(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. 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.
5. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. 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.
8. 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)