Technical Data Sheet

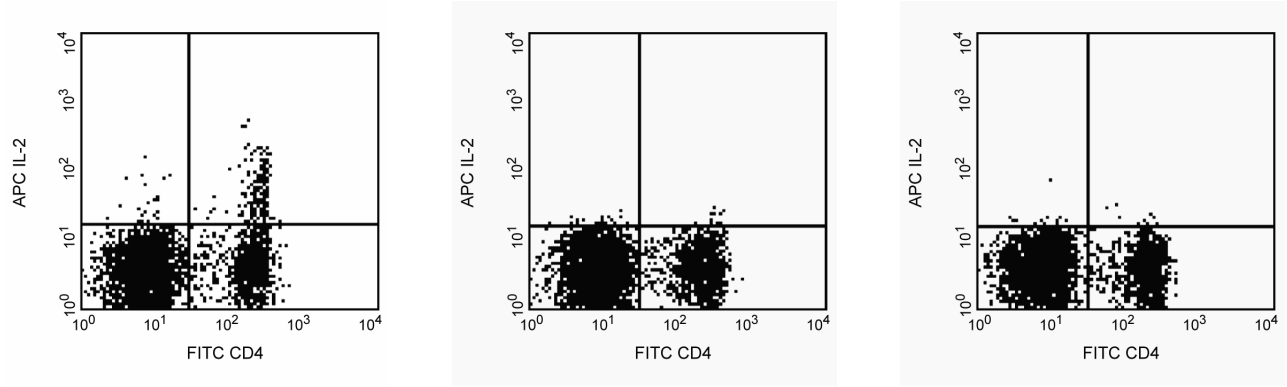
APC Rat Anti-Mouse IL-2

Product Information

Material Number:	**562041**
Alternate Name:	Il2; Interleukin-2; T-cell growth factor; TCGF
Size:	25 µg
Concentration:	0.2 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 monoclonal antibody specifically binds to mouse interleukin-2 (IL-2). IL-2 is a multifunctional cytokine that plays pivotal roles in immunity and tolerance. It is produced by activated T cells. IL-2 effects the activation, growth, proliferation and/or differentiation of various cell types including T and B lymphocytes and their precursors, LAK cells, NK cells, and monocytes/macrophages. IL-2 mediates its biological activities by binding to IL-2 receptor compleses. The intermediate affinity IL-2R is comprised of IL-2Rβ (CD122) and common gamma chain (γc; CD132) subunits whereas the high-affinity IL-2R is comprised of IL-2Rα (CD25), IL-2Rβ and γc subunits. The JES6-5H4 monoclonal antibody binds to IL-2 and neutralizes its biological activity.



Expression of IL-2 by stimulated CD4+ and CD4- BALB/c spleen cells. *Splenocytes from 6 month old BALB/C mice were stimulated for 15 hr with hamster anti-mouse CD3 (25 µg/ml, clone 145-2C11, Cat. No. 553057) and hamster anti-mouse CD28 (2 µg/ml, clone 37.51, Cat. No. 553294) antibodies in the presence of BD GolgiPlug™ (Cat. No. 555029). The splenocytes were harvested, stained with 0.06 µg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.25 µg of APC-conjugated rat anti-mouse IL-2 antibody (APC-JES6-5H4, Cat. No. 562041) by using the BD Pharmingen staining protocol (left panel). To demonstrate specificity of staining, the staining by APC-JES6-5H4 was blocked by each of the following: 1) preincubation of the conjugated antibody with recombinant mouse IL-2 (0.25 µg, Cat. No. 550069; middle panel) and by 2) preincubation of the fixed/permeabilized cells with unlabelled JES6-5H4 mouse antibody (5.0 µg; Cat. No. 554425) prior to staining with the APC-JES6-5H4 (right panel). 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. 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:

Immunofluorescent Staining for Flow Cytometric Analysis: The APC JES6-5H4 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-2 producing cells within mixed cell populations. For optimal immunofluorescent staining for flow cytometric analysis, the anti-cytokine antibody should be titrated. For specific methodology, visit the protocols section of our website, www.bdbiosciences.com. A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the APC-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

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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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
556924	APC 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	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. 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. 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.
6. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
7. An isotype control should be used at the same concentration as the antibody of interest.

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)