APC-Cy™7 Rat Anti-Mouse IL-2

Product Information

**Material Number:** 560547

**Alternate Name:** IL2; Interleukin-2; T-cell growth factor; TCGF

**Size:** 50 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 protein stabilizer and ≤0.09% sodium azide.

**Description**

The JES6-5H4 monoclonal antibody specifically binds to mouse interleukin-2 (IL-2), a multifunctional cytokine that plays pivotal roles in immunity and tolerance. It is produced by activated T cells and affects 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 complexes. 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.

**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 with APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

**Application Notes**

**Application**

| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

**Flow cytometry:** 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. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant mouse IL-2 (Cat. No. 550069) or (2) purified JES6-5H4 antibody (Cat. No. 554425), prior to staining.

**Cell Preparation:** Investigators not wishing to utilize MiCK-1 cells may alternatively prepare mouse splenocytes (e.g BALB/c) stimulated for 4-6 hours with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng/mL, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ Cat. No. 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

**Flow cytometric analysis for IL-2 in activated mouse splenocytes.** Mouse Intracellular Cytokine-1 positive control cells MiCK-1 (Cat. No. 554652) are activated mouse splenocytes prepared in the presence of a protein transport inhibitor (Cat. No. 555029). Fixed and permeabilized MiCK-1 cells were stained either with a APC-Cy™7 Rat IgG2b, κ isotype control (Cat. No. 552773, left panel) or with the APC-Cy™7 Rat Anti-Mouse IL-2 antibody (Cat. No. 560547, right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.
Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>552773</td>
<td>APC-Cy™7 Rat IgG2b κ Isotype Control</td>
<td>0.1 mg</td>
<td>A95-1</td>
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<tr>
<td>550069</td>
<td>Recombinant Mouse IL-2</td>
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<td>554425</td>
<td>Purified Rat Anti-Mouse IL-2</td>
<td>0.1 mg</td>
<td>JES6-5H4</td>
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<td>554652</td>
<td>MiCK-1 Mouse Cytokine Positive Control Cells</td>
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<td>555029</td>
<td>Protein Transport Inhibitor (Containing Brefeldin A)</td>
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<tr>
<td>555028</td>
<td>BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)</td>
<td>250 Tests</td>
<td>(none)</td>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
7. Cy is a trademark of GE Healthcare.
8. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References