Technical Data Sheet

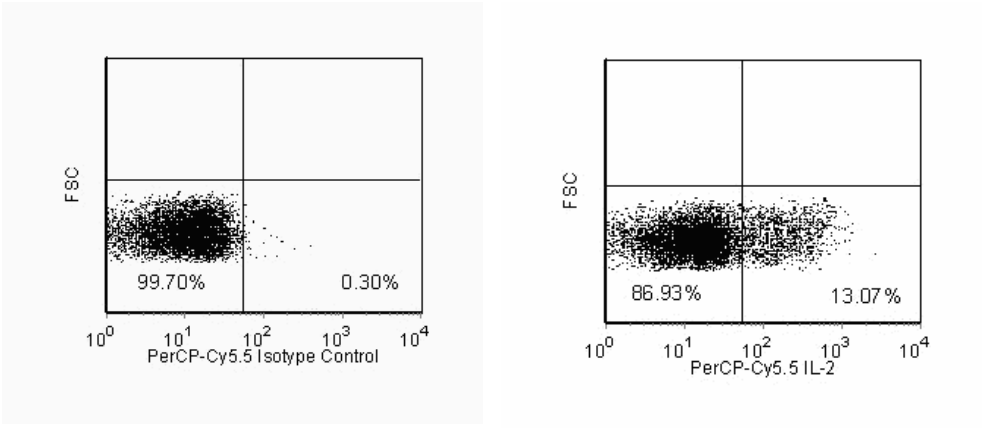
PerCP-Cy™5.5 Rat Anti-Mouse IL-2

Product Information

Material Number:	**560544**
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 ≤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.



Flow cytometric analysis for IL-2 in activated mouse splenocytes. *Mouse Intracellular Cytokine-1 positive control cells (MiCK-1) offered by BD Biosciences as MN 554652, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-1 cells were stained either with a PerCP-Cy™5.5 Rat IgG2b, κ isotype control (left panel) or with the PerCP-Cy™5.5 Rat Anti-Mouse IL-2 antibody (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.*

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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

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) unlabeled 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, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
550764	PerCP-Cy™5.5 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
550069	Recombinant Mouse IL-2	20 µg	(none)
554425	Purified Rat Anti-Mouse IL-2	0.1 mg	JES6-5H4
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)

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. 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.
4. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
7. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
9. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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