Technical Data Sheet

APC Rat Anti-Human IL-10

Product Information

Material Number:	**554707**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	JES3-19F1
Immunogen:	Recombinant Human IL-10
Isotype:	Rat IgG2a
Reactivity:	QC Testing: Human Tested in Development: Viral
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The JES3-19F1 antibody reacts with human (IL-10). The immunogen used to generate the JES3-19F1 hybridoma was recombinant human IL-10 expressed in COS cells. This is a neutralizing antibody. This antibody also reacts with viral IL-10.

Preparation and Storage

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.
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:

Immunofluorescent Staining and Flow Cytometric Analysis: The JES3-19F1 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-10 producing cells within mixed cell populations. The APC-conjugated JES3-19F1 antibody (Cat. No. 554707) is especially suitable for these studies (see image). For optimal immunofluorescent staining and flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocol section or the chapter on intracellular staining in the Immune Function Handbook.

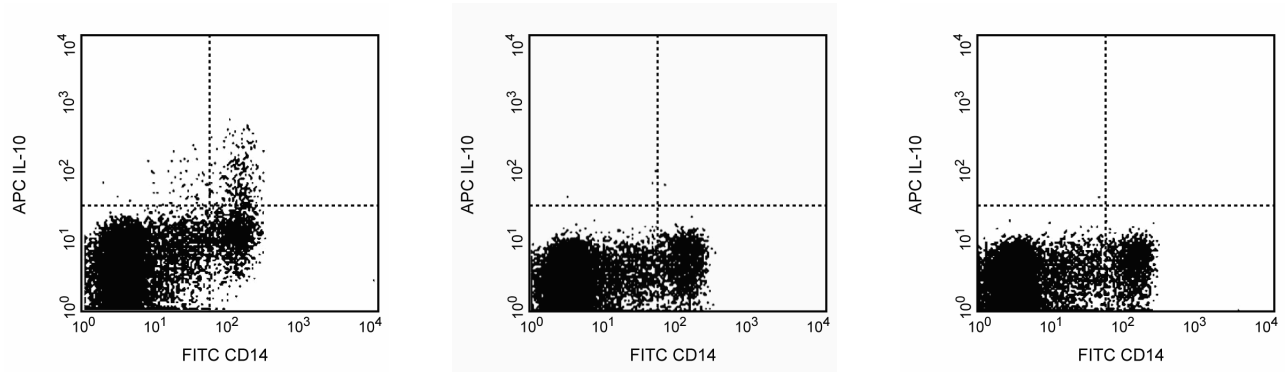
A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated JES3-19F1 antibody with ligand (e.g., recombinant human IL-10; Cat. No. 554611) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled JES3-19F1 antibody (Cat. No. 554705) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is PE-R35-95 (Cat. No. 554690); use at comparable concentrations to the antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**



Expression of IL-10 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 24 hours with LPS (1 µg/ml final concentration) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were harvested, stained with FITC-mouse anti-human CD14 antibody (FITC-M5E2, Cat. No. 555397), fixed, permeabilized, and subsequently stained with 0.007 µg of APC-rat anti-human IL-10 antibody (APC-JES3-19F1, Cat. No. 554707) following the BD Bioscience staining protocol (left panel). The data reflect gating on monocytes, based on forward and side scatter. To demonstrate specificity of staining, the binding of APC-JES3-19F1 was blocked by the preincubation of the conjugated antibody with recombinant human IL-10 (0.25 µg, Cat. No. 554611; middle panel), and by preincubation of the fixed/permeabilized cells with unlabelled JES3-19F1 antibody (10 µg, Cat. No. 554705; right panel) prior to staining with the APC-JES3-19F1 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) and unlabelled antibody (right panel) blocking specificity controls. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNE or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.*

Suggested Companion Products

Catalog Number	Name	Size	Clone
554690	APC Rat IgG2a κ Isotype Control	0.1 mg	R35-95
555062	HiCK-2 Human Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(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.

References

Andersson EC, Christensen JP, Marker O, Thomsen AR. Changes in cell adhesion molecule expression on T cells associated with systemic virus infection. *Immunology.* 1994; 152(3):1237-1245. (Clone-specific)

D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med.* 1993; 178(3):1041-1048. (Clone-specific: Neutralization)

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: Blocking)