Technical Data Sheet

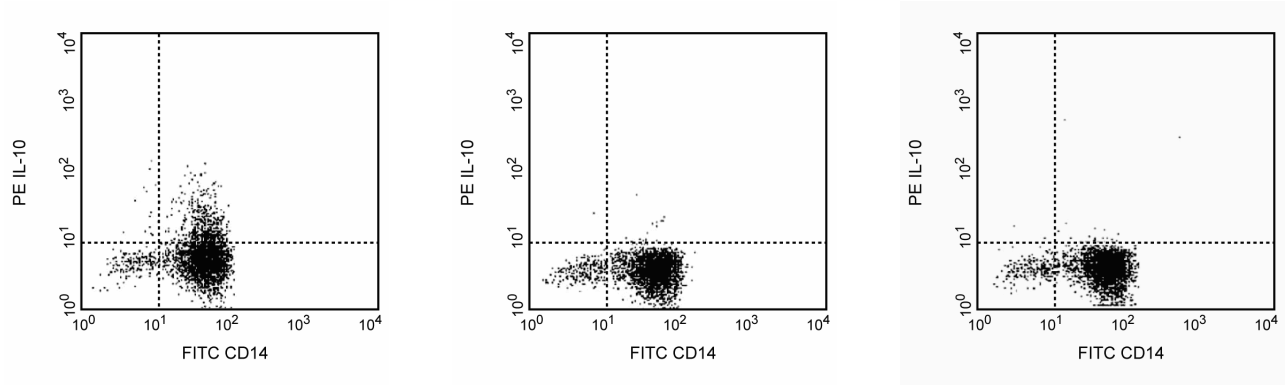
PE Rat Anti-Human IL-10

Product Information

Material Number:	**562035**
Size:	25 tests
Vol. per Test:	20 µl
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 BSA and ≤0.09% sodium azide.

Description

The JES3-19F1 antibody reacts with human interleukin-10 (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.



Expression of IL-10 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 24 hr with LPS (1.0 ug/ml final concentration) in the presence of BD GolgiStop™ (Cat. No. 554724; 2 µM final concentration). The PBMC were harvested, stained with FITC Mouse anti-Human CD14 antibody (Clone M5E2), fixed, permeabilized, and subsequently stained with 20 µl of PE Rat anti-Human IL-10 antibody following the Usage section below (see image, left panel). The data reflects gating on monocytes, based on forward and side scatter. To demonstrate specificity of staining, PE-JES3-19F1 binding 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 (5.0 µg, right panel) prior to staining with the PE-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.*

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
ELISA Capture	Tested During Development
Neutralization	Tested During Development

Recommended Assay Procedure:

1. Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated JES3-19F1 antibody can be used for multicolor flow cytometric analysis to identify and enumerate IL-10-producing cells within mixed cell populations (see image). This 25 Test Size formulation of the PE-conjugated JES3-19F1 antibody has been pre-titrated to assure effective intracellular detection of human IL-10 using 20 µl/1 x 10^6 cells.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated JES3-19F1 antibody with ligand (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. 554704/554705) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

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Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the USAGE section below.

USAGE

1. Resuspend 1 x 10^6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X Perm/Wash Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (at RT or 4°C).
3. Wash twice in 100 µl of 1X Perm/Wash Buffer.

2. ELISA Capture: The purified JES3-19F1antibody is useful as a capture antibody for a sandwich ELISA for specifically measuring human IL-10 protein levels. Purified JES3-19F1 antibody can be paired with the biotinylated JES3-12G8 antibody (Cat. No. 554499) as the detection antibody and with recombinant human IL-10 protein (Cat. No. 554611) as the standard. For testing IL-10 in serum or plasma, our OptEIA™ set (Cat. No. 555134) is recommended.

3. Neutralization: The NA/LE™ format of clone JES3-19F1 (Cat. No. 559330) is useful for neutralization of human IL-10 bioactivity. A suitable NA/LE™ rat IgG2a isotype control to match the NA/LE JES3-19F1 antibody is the R35-95 antibody, (Cat. No. 554687).

Suggested Companion Products

Catalog Number	Name	Size	Clone
555062	HiCK-2 Human Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
559317	PE Rat IgG2a κ Isotype Control	100 tests	R35-95
554723	Perm/Wash Buffer	100 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
561712	FITC Mouse Anti-Human CD14	25 tests	M5E2
554655	Fixation Buffer	100 ml	(none)
554611	Recombinant Human IL-10	5 µg	(none)
554704	Purified Rat Anti-Human IL-10	0.1 mg	JES3-19F1

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)