Technical Data Sheet

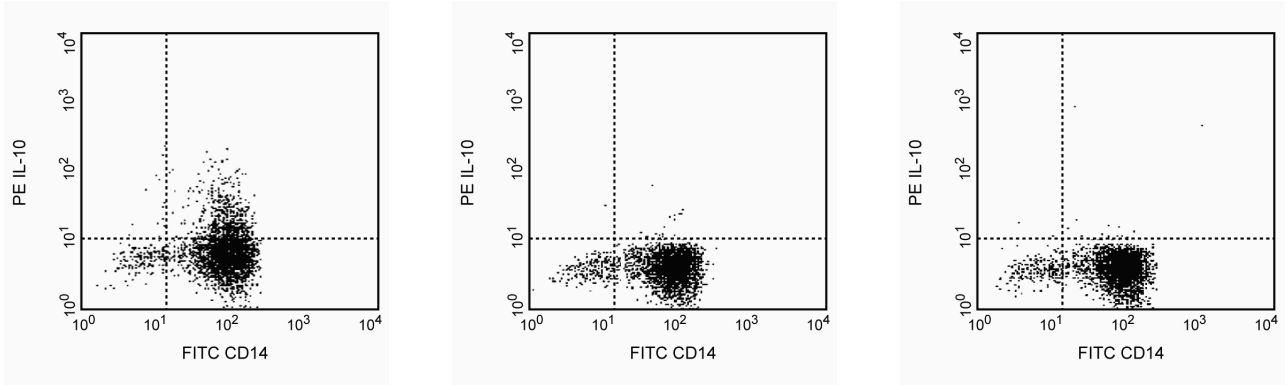
Purified Rat Anti-Human IL-10

Product Information

Material Number:	**554704**
Size:	0.1 mg
Concentration:	0.5 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.



Expression of IL-10 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 24 hours with LPS (1.0 ug/ml) in the presence of GolgiStop™ (Cat. No. 554724; 2 µM final concentration). The PBMC were harvested, stained with FITC-mouse anti-human CD14 antibody (FITC-M5E2, Cat. No. 555397), fixed, permeabilized, and subsequently stained with 0.25 µg of PE-rat anti-human IL-10 antibody (PE-JES3-19F1, Cat. No. 554706) following Pharmingen's staining protocol (see image, left panel). The data reflects gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, the binding of the PE-JES3-19F1 antibody was blocked by the preincubation of the conjugated antibody with recombinant human IL-10 (0.5 mg, Cat. No. 554611; middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled JES3-19F1 antibody (10 mg, Cat. No. 554704; 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 unlabeled antibody (right panel) blocking specificity controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

Application Notes

Application

ELISA Capture	Routinely Tested
Neutralization	Tested During Development
Intracellular block/flow cytometry	Tested During Development

Recommended Assay Procedure:

1. Blocking Control for Intracellular Staining: The purified JES3-19F1 antibody can be used as a blocking control to demonstrate specificity of IL-10 staining by PE-JES3-19F1 (Cat. No. 554706). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1-10 µg of unlabeled JES3-19F1 antibody (Cat. No. 554704) for 20 minutes at 4°C, prior to staining with PE-JES3-19F1 antibody (e.g., 0.1 -0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

Please note that this application is not routinely tested at BD Biosciences.

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2. ELISA Capture: The purified JES3-19F1 antibody 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. 554703) 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
554706	PE Rat Anti-Human IL-10	0.1 mg	JES3-19F1
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554703	Purified NA/LE Rat Anti-Human IL-10	0.5 mg	JES3-19F1
554705	Purified Rat Anti-Human IL-10	0.5 mg	JES3-19F1
554611	Recombinant Human IL-10	5 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 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. 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)