Technical Data Sheet

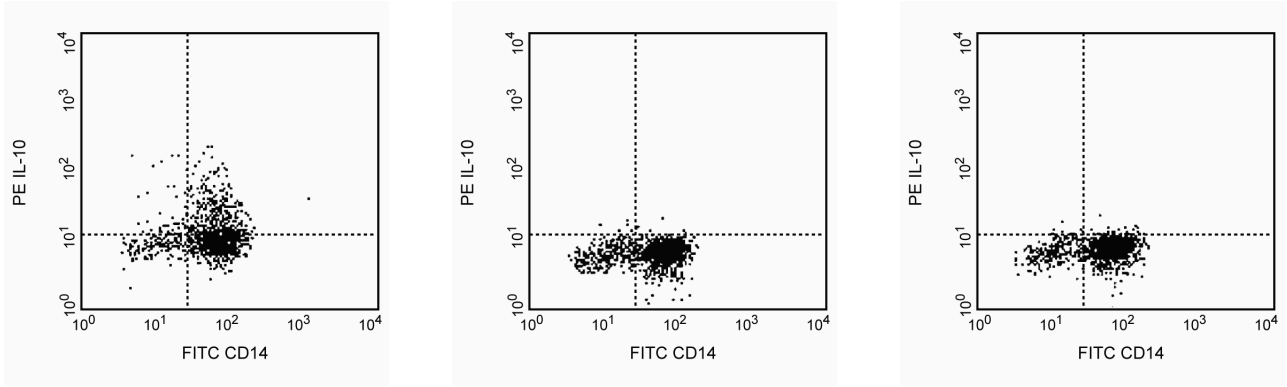
Purified Rat Anti-Human and Viral IL-10

Product Information

Material Number:	**554496**
Alternate Name:	CSIF; Cytokine synthesis inhibitory factor; TGIF
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	JES3-9D7
Immunogen:	Recombinant Human IL-10
Isotype:	Rat IgG1
Reactivity:	QC Testing: Human Tested in Development: Viral
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The JES3-9D7 monoclonal antibody specifically reacts with human IL-10 (Interleukin-10) and viral IL-10. The immunogen used to generate the JES3-9D7 hybridoma was recombinant human IL-10 expressed by COS cells. IL-10 is also known as CSIF (Cytokine synthesis inhibitory factor) and (TGIF) T-cell growth inhibitory factor. IL-10 is expressed by various cell types including activated monocytes, macrophages, dendritic cells, mast cells, granulocytes, and lymphocytes. IL-10 is a pleiotropic cytokine that can downregulate proinflammatory immune responses, such as Th1-like responses, while promoting other responses including B cell proliferation and antibody production.



Expression of IL-10 by stimulated CD14+ human monocytes. *Human PBMC were stimulated for 24 hours with LPS (1.0 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 0.25 µg of PE-rat anti-human IL-10 antibody (PE-JES3-9D7, Cat. No. 554498) following Pharmingen's staining protocol (left panel). The data reflect gating on monocytes, based on forward and side light scatter. To demonstrate specificity of staining, the binding of PE-JES3-9D7 was blocked by the preincubation of the conjugated antibody with recombinant human IL-10 (0.5 µg; Cat. No. 554611; middle panel), and by preincubation of the fixed/permeabilized cells with the unlabeled JES3-9D7 antibody (5.0 µg; Cat. No. 554496; right panel) prior to staining with the PE-JES3-9D7 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

Store undiluted at 4°C.

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

Application Notes

Application

ELISA Capture	Routinely Tested
Intracellular block/flow cytometry	Tested During Development
Western blot	Reported

Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified JES3-9D7 antibody can be used as a blocking control to demonstrate specificity of IL-10 staining by PE-JES3-9D7 antibody (Cat. No. 554498). To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1-10 µg of unlabeled JES3-9D7 antibody (Cat. No. 554496) for 20 minutes at 4°C, prior to staining with PE-JES3-9D7 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.

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Neutralization: The NA/LE format of the JES3-9D7 antibody (Cat. No. 554495) is useful for neutralization of human or viral IL-10 bioactivity. A suitable NA/LE™ rat IgG1 isotype control to match the NA/LE™ JES3-9D7 antibody is the R3-34 antibody (Cat. No. 554682).

Western Blot: The JES3-9D7 antibody (e.g., Cat. No. 554496) has been reported to be useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554498	PE Rat Anti-Human and Viral IL-10	0.1 mg	JES3-9D7
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
555062	HiCK-2 Human Cytokine Positive Control Cells	1 mL	(none)
554499	Biotin Anti-Human and Viral IL-10	0.5 mg	JES3-12G8
554611	Recombinant Human IL-10	5 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. *Curr Protoc Immunol.* 2001; 1:6.20-6.21. (Clone-specific: ELISA)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific: ELISA)

Burdin N, Peronne C, Banchereau J, Rousset F. Epstein-Barr virus transformation induces B lymphocytes to produce human interleukin 10. *J Exp Med.* 1993; 177(2):295-304. (Clone-specific: ELISA)

Gotlieb WH, Abrams JS, Watson JM, Velu TJ, Berek JS, Martinez-Maza O. Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. *Cytokine.* 1992; 4(5):385-390. (Clone-specific: ELISA)

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)

Yssel H, De Waal Malefyt R, Roncarolo MG, et al. IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. *J Immunol.* 1992; 149(7):2378-2384. (Clone-specific: ELISA)

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