Technical Data Sheet

PE-Cy7 Rat Anti-Human IL-10

Product Information

Material Number:	**567407**
Alternate Name:	Interleukin-10; CSIF; Cytokine synthesis inhibitory factor; TGIF
Size:	100 Tests
Vol. per Test:	5 µl/test
Clone:	JES3-19F1
Immunogen:	Recombinant Human IL-10
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The JES3-19F1 monoclonal antibody specifically recognizes human Interleukin-10 (IL-10) that is encoded by *IL10*. IL-10 is also known as Cytokine Synthesis Inhibitory Factor (CSIF), B cell-derived T cell growth factor (B-TCGF), and T-cell growth inhibitory factor (TGIF). The JES3-19F1 antibody crossreacts with ebvIL-10 protein, the Epstein-Barr viral IL-10 homolog (viral IL-10 or vIL-10) encoded by the *BCRF1* gene. IL-10 is produced by a variety of cells such as some activated T cells and B cells including regulatory T cells (Treg) and B cells (Breg), monocytes and macrophages, dendritic cells (DC), keratinocytes, and mast cells. IL-10 is a multifunctional cytokine that can downregulate immune and proinflammatory responses. IL-10 can act to reduce expression of major histocompatibility complex class II antigens, costimulatory molecules, or proinflammatory cytokines including IL-1β, IL-2, IL-3, IL-12, IFN-γ, TNF or GM-CSF expressed by activated monocytes, macrophages, dendritic cells (DC), natural killer (NK) cells, or T cells. IL-10 has been shown to play a role in chronic viral infections. IL-10 can also enhance B cell survival, proliferation, and differentiation to become antibody-producing cells. The JES3-19F1 antibody reportedly neutralizes the biological activity of human IL-10 and ebvIL-10. IL-10 mediates its biological activities by signaling through a heterotetrameric receptor complex composed of the type II cytokine receptor subunits CD210a (IL-10 Rα) and CD210b (IL-10 Rβ).

PE-Cy7 dye is a part of the BD PE family of dyes. This tandem fluorochrome is comprised of a R-Phycoerythrin (PE) donor that has excitation maxima (Ex Max) of 496-nm and 566-nm and an acceptor dye, Cy™7, with an emission maximum (Em Max) at 781-nm. PE can be excited by the Blue (488-nm), Green (532-nm) and yellow-green (561-nm) lasers and detected using an optical filter centered near 781 nm (e.g., a 760/60-nm bandpass filter). The donor dye can be excited by the Blue (488-nm), Green (532-nm) and yellow-green (561-nm) lasers and the acceptor dye can be excited by the Red (627–640-nm) laser resulting in cross-laser excitation and fluorescence spillover. Please ensure that your instrument's configurations (lasers and optical filters) are appropriate for this dye.

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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

BD® CompBeads can be used as surrogates to assess fluorescence spillover (compensation). When fluorochrome conjugated antibodies are bound to BD® CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells , resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cell and BD® CompBeads to ensure that BD® CompBeads are appropriate for your specific cellular application.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
552784	PE-Cy™7 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
2. 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).

BD Biosciences

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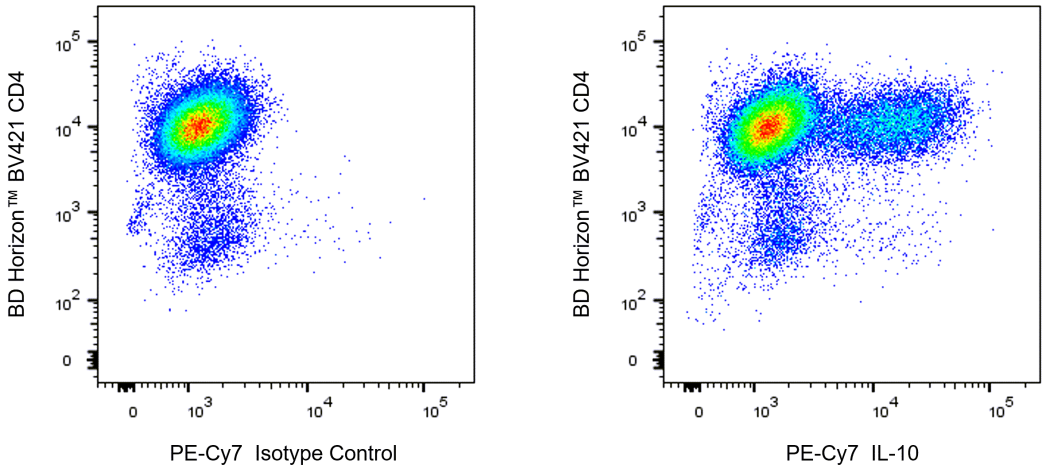
For country contact information, visit **bdbiosciences.com/contact**

3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
8. An isotype control should be used at the same concentration as the antibody of interest.
9. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
10. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
11. Cy is a trademark of Global Life Sciences Solutions Germany GmbH or an affiliate doing business as Cytiva.



Two-color flow cytometric analysis of IL-10 expressed in stimulated human lymphocytes. *CD4+ peripheral blood mononuclear cells were obtained by panning using plate-bound Purified NA/LE Mouse Anti-Human CD4 antibody (Cat. No. 555343). The cells were cultured (5 d) with plate-bound NA/LE Mouse Anti-Human CD3 (Cat. No. 555329; 10 µg/ml, coated overnight at 4°C) and soluble NA/LE Mouse Anti-Human CD28 (Cat. No. 555725; 1 µg/ml) antibodies plus recombinant Human IL-2 (Cat. No. 554603; 20 ng/ml) and IL-4 (Cat. No. 554605; 40 ng/ml) proteins. The cells were restimulated (5 h) with Phorbol 12-Myristate 13-Acetate (PMA; Sigma P8139; 50 ng/ml) and Ionomycin (Sigma I9657; 1 µg/ml) and BD GolgiStop™ Protein Transport Inhibitor (Cat. No. 554724). The cells were harvested, fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), and permeabilized with and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with BD Horizon™ BV421 Mouse Anti- Human CD4 antibody (Cat. No. 565997/566907) and with either PE-Cy7 Rat IgG2a, κ Isotype Control (Cat. No. 552784; Left Plot) or PE-Cy7 Mouse Anti-Human IL-10 antibody (Cat. No. 567407/567408; Right Plot). The bivariate pseudocolor density plot showing the correlated expression of IL-10 (or Ig Isotype control staining) versus CD4 was derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software.*

References

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