Technical Data Sheet

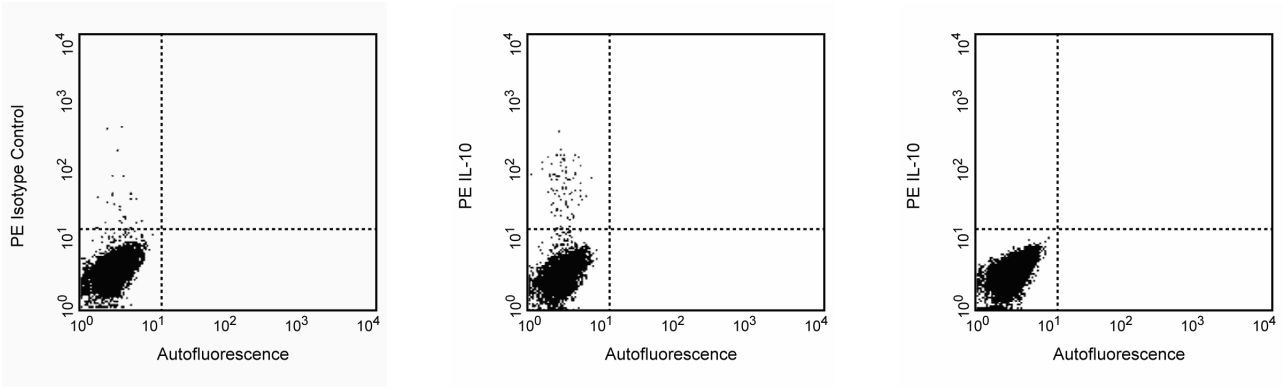
PE Rat Anti-Human and Viral IL-10

Product Information

Material Number:	**559337**
Size:	100 tests
Vol. per Test:	20 µl
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 BSA and ≤0.09% sodium azide.

Description

The JES3-9D7 antibody reacts with human IL-10 and viral IL-10. The immunogen used to generate the JES3-9D7 hybridoma was recombinant human IL-10 expressed in COS cells.



Expression of IL-10 by stimulated human monocytes. *Human PBMC were stimulated overnight with 1.0 µg/ml LPS in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were then harvested fixed, permeabilized, and subsequently stained with 20 µl of PE-rat anti-human IL-10 antibody (Cat. No. 554498) following the Usage section above and BD Pharmingen staining protocol (middle 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 fixed/permeabilized cells with the unlabeled JES3-9D7 antibody (5.0 µg final concentration; 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 staining profile using 20µl of PE-R3-34 isotype control antibody (Cat. No. 559318, left panel) and verified using the unlabeled antibody blocking specificity control.*

Preparation and Storage

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.
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 PE-conjugated JES3-9D7 antibody can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-10-producing cells within mixed cell populations (see image). This 100 Test Size formulation of the PE-conjugated JES3-9D7 antibody has been pre-titrated to assure effective intracellular detection of human and viral 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 JES3-9D7 antibody with ligand (recombinant human IL-10; Cat. No. 554611) prior to staining, or 2) pre-block the

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fixed/permeabilized cells with unlabeled JES3-9D7 antibody (Cat. No. 554496) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-R3-34 (Cat. No. 559318). For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Important Note: This pretitered 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 (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
559318	PE Rat IgG1, κ Isotype Control	100 tests	R3-34
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	1.0 ml	(none)

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1995:6.20-6.21. (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: Blocking, Flow cytometry)

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)