Technical Data Sheet

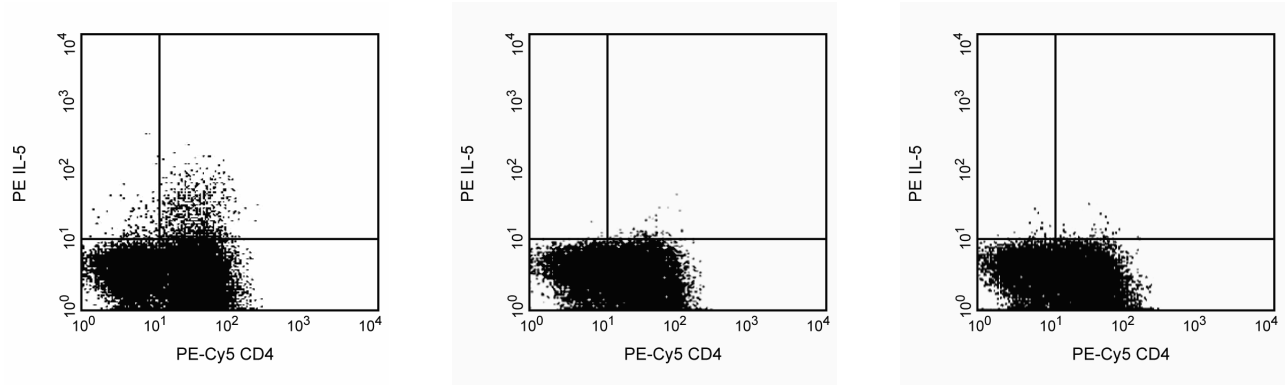
PE Rat Anti-Human IL-5

Product Information

Material Number:	**559332**
Size:	100 tests
Vol. per Test:	20 µl
Clone:	JES1-39D10
Immunogen:	Recombinant human IL-5
Isotype:	Rat IgG2a
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The JES1-39D10 antibody reacts with human interleukin-5 (IL-5). The immunogen used to generate the JES1-39D10 hybridoma was COS-expressed recombinant human IL-5. This is a neutralizing antibody.



Detection of IL-5 expression by stimulated human CD4+ T cells. *Isolated human CD4+ cells were stimulated with immobilized anti-human CD3 (1 µg/ml final concentration; UCHT1, Cat. No. 555329), soluble anti-human CD28.2 antibody (20 ng/ml final concentration; Cat. No. 555725), recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 555605) and recombinant human IL-4 (10 ng/ml final concentration; Cat. No. 554603) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant human IL-4 for 3 days. The cells were then harvested and restimulated for 6 hours with PMA (Sigma, Cat. #P-8139) and calcium ionophore A23187 (Sigma, Cat. #C-9275) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). Finally, the cells were harvested, stained with 0.25 µg of PE-Cy5-anti-CD4 (PE-Cy5-RPA-T4, Cat. No. 555348), fixed, permeabilized, and subsequently stained with 20 µl PE-conjugated rat anti-human IL-5 (PE-JES1-39D10, Cat. No. 559332) by following the usage section above and Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-JES1-39D10 antibody was blocked by the preincubation of the conjugated antibody with recombinant human IL-5 (0.1 µg; Cat. No. 554606; middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled JES1-39D10 antibody (5 µg/Cat. No. 554487; right panel) prior to staining with the PE-JEs1-39D10 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control and verified with the recombinant cytokine blocking and unlabeled blocking specificity controls.*

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 JES1-39D10 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate human IL-5 producing cells within mixed cell populations. The PE-conjugated JES1-39D10 antibody is especially suitable for these experiments (see Figure). This 100 Test Size formulation of the PE-conjugated JES1-39D10 antibody has been pretitrated to assure effective intracellular detection of human IL-5 using 20 µl/1 x 10e6 cells. 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.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the PE-JES1-39D10 antibody with excess ligand (e.g., human IL-5, Cat. No. 554606) prior to staining, or 2) pre-block paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled JES1-39D10 antibody (e.g., Cat. No. 554488) prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG2a isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is also available in a 100 Test Size formulation PE-R35-95 (Cat. No. 559317).

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.

USAGE

1. Resuspend 1 x 10e6 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 room temperature 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
554606	Recombinant Human IL-5	5 µg	(none)
559317	PE Rat IgG2a κ Isotype Control	100 tests	R35-95
554723	Perm/Wash Buffer	100 ml	(none)
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
554603	Recombinant Human IL-2	10 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555348	PE-Cy™5 Mouse Anti-Human CD4	100 tests	RPA-T4
554605	Recombinant Human IL-4	5 µg	(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. 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

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)

Abrams JS, Silver J, Van Dyke R, Gleich G. Eosinophil-active cytokines in human disease: development and use of monoclonal antibodies to IL-3, IL-5, GMCSF. In: Kay A and Gleich G, ed. *Eosinophils in Allergy and Inflammation.* 1994:133-157. (Clone-specific: ELISA)

Butterfield JH, Leiferman KM, Abrams J, et al. Elevated serum levels of interleukin-5 in patients with the syndrome of episodic angioedema and eosinophilia. *Blood.* 1992; 79(3):688-692. (Clone-specific)

Elson LH, Nutman TB, Metcalfe DD, Prussin C. Flow cytometric analysis for cytokine production identifies T helper 1, T helper 2, and T helper 0 cells within the human CD4+CD27- lymphocyte subpopulation. *J Immunol.* 1995; 154(9):4294-4301. (Clone-specific: Flow cytometry)

Jung T, Schauer U, Rieger C, et al. Interleukin-4 and interleukin-5 are rarely co-expressed by human T cells. *Eur J Immunol.* 1995; 25(8):2413-2416. (Clone-specific: Flow cytometry)

Limaye AP, Abrams JS, Silver JE, et al. Interleukin-5 and the posttreatment eosinophilia in patients with onchocerciasis. *J Clin Invest.* 1991; 88(4):1418-1421. (Clone-specific)

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: IC/FCM Block)