

Technical Data Sheet

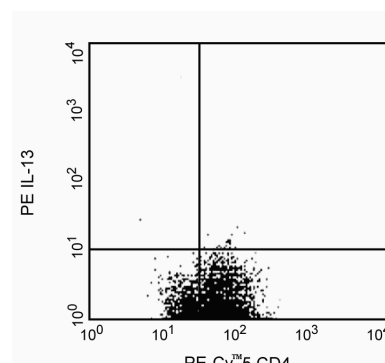
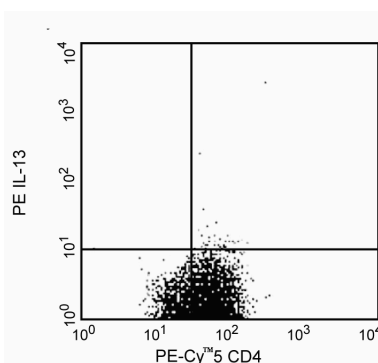
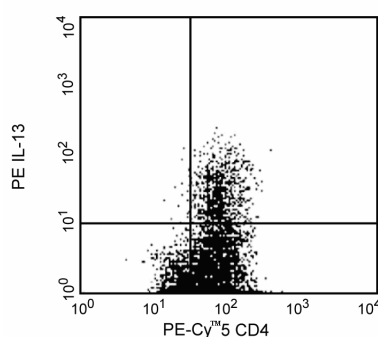
PE Rat Anti-Human IL-13

Product Information

Material Number:	562039
Alternate Name:	Interleukin-13; NC30; ALRH; BHR1; P600
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	JES10-5A2
Immunogen:	Human Recombinant IL-13
Isotype:	Rat IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The JES10-5A2 monoclonal antibody specifically binds to human interleukin-13, IL-13. IL-13 is produced by activated T cells, mast cells and NK cells. IL-13 regulates IgE production by B cells and can suppress the cytotoxic activity of macrophages and their production of inflammatory mediators. The immunogen used to produce the JES10-5A2 hybridoma was COS-expressed recombinant human IL-13. This is a neutralizing antibody.



Expression of IL-13 by stimulated human CD4+ cells. Isolated human CD4+ cells were stimulated with immobilized anti-human CD3 antibody (UCHT1, 10 µg/ml for plate coating, Cat. No. 555329), soluble anti-human CD28 antibody (20 ng/ml, Cat. No. 555725), recombinant human IL-2 (10 ng/ml, Cat. No. 554603) and recombinant human IL-4 (10 ng/ml, Cat. No. 554605) for 2 days. The cells were washed and subsequently cultured in medium containing IL-2 and IL-4 for 3 days. Finally, the cells were harvested and re-stimulated for 6 hr with PMA (Sigma, Cat. #P-8139; 50 ng/ml), calcium ionophore A23187 (Sigma, Cat. #C-9275; 250 ng/ml) in the presence of GolgiStop™ (2 µM final concentration, Cat. No. 554724). The cells were stained with PE-Cy5™5 Mouse anti-Human CD4 (Cat. No. 555348), fixed, permeabilized, and subsequently stained with 0.25 µg of PE-Rat anti-Human IL-13 antibody by using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-JES10-5A2 antibody was blocked by the preincubation of the conjugated antibody with excess recombinant human IL-13 (middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled JES10-5A2 antibody (5 µg, Cat. No. 554570; right panel) prior to staining with the PE Rat anti-Human IL-13 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 antibody blocking specificity controls.

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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)

Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated JES10-5A2 antibody can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-13 producing cells within mixed cell populations (see Figure). For optimal immunofluorescent staining and flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit our website, 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 JES10-5A2 antibody with ligand (e.g., human IL-13) prior to staining, or 2) pre-block paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled JES10-5A2 antibody (e.g., Cat. No. 554570) 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 IgG1 isotype control immunoglobulin for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is the R3-34 antibody (Cat. No. 554685); use at comparable concentrations to antibody of interest (e.g., $\leq 0.5 \mu\text{g mAb}/1$ million cells).

ELISA Capture: The purified JES10-5A2 (Cat. No. 554570) is useful as a capture antibody for a sandwich ELISA for specifically measuring human IL-13 protein levels. Purified JES10-5A2 antibody can be paired with the biotinylated B69-2 antibody (Cat. No. 555054), with recombinant human IL-13 protein as the standard.

Neutralization: The BD NA/LE™ JES10-5A2 antibody (Cat. No. 554568) is useful for neutralization of human IL-13 bioactivity. A suitable BD NA/LE™ isotype control to match the JES10-5A2 antibody is the R3-34 antibody (Cat. No. 554682).

WB: The JES10-5A2 antibody has been reported to be useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
554603	Recombinant Human IL-2	10 μg	(none)
554605	Recombinant Human IL-4	5 μg	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554570	Purified Rat Anti-Human IL-13	0.5 mg	JES10-5A2
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555348	PE-Cy™5 Mouse Anti-Human CD4	100 tests	RPA-T4

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.
5. An isotype control should be used at the same concentration as the antibody of interest.
6. Cy is a trademark of Amersham Biosciences Limited.

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)

McKenzie A, Zurawski G. Measurement of IL-13. In: Coligan, Kruisbeek, Shevak, Strober, ed. *Current Protocols in Immunology*. New York: John Wiley & Sons; 1994:18-19. (Clone-specific: ELISA, 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: IC/FCM Block)