Technical Data Sheet

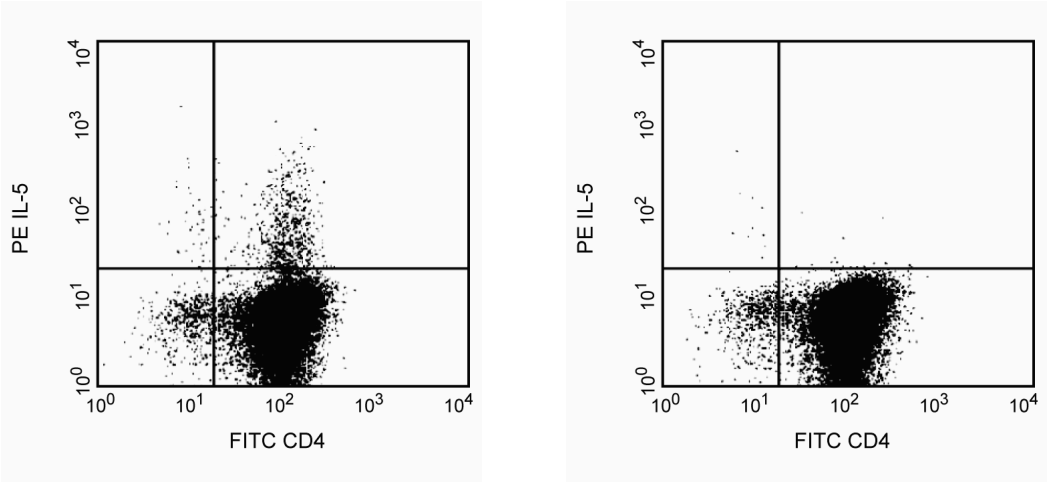
PE Rat Anti-Mouse/Anti-Human IL-5

Product Information

Material Number:	**562049**
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	TRFK5
Immunogen:	Mouse Semi-Purified T-Cell Clone Supernatant
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TRFK5 antibody reacts with mouse interleukin-5 (IL-5) and cross-reacts with human IL-5. The TRFK5 antibody has been reported to cross react with IL-5 from rhesus monkey. This is a neutralizing antibody.



Expression of IL-5 by stimulated mouse CD4+ T cells. *Purified splenic CD4+ cells from 6-month old BALB/c mice were stimulated with plate-bound anti-CD3 (clone 145-2C11, Cat. No. 553057 at 25 µg/ml) and soluble anti-mouse CD28 (clone 37.51, Cat. No. 553294 at 2 µg/ml) for 2 days in culture together with recombinant mouse IL-2 (10 ng/ml, Cat. No. 550069) and recombinant mouse IL-4 (0.5 ng/ml, Cat. No. 550067), followed by a 3 day incubation with only recombinant IL-2 and IL-4. This was followed by a 5 hour stimulation with plate-bound anti-CD3 (25 µg/ml) and anti-mouse CD28 (2 µg/ml) in the presence of BD GolgiStop™ (Cat. No. 554724). The cells were harvested, stained with 0.05 µg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.12 µg of PE-conjugated rat anti-mouse/human IL-5 antibody (PE-TRFK5, Cat. No. 554395) by using the BD Pharmingen staining protocol (left panel). The binding of PE-TRFK5 was blocked by preincubation of the conjugate with recombinant mouse IL-5 (0.25 µg; Cat. No. 554581; right panel). The quadrant markers for the bivariate dot plots were set based on the unstained cell control.*

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:

For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). A suitable rat IgG1 isotype control assessing the level of background staining on fixed/permeabilized or human cells is PE-R3-34 (Cat. No. 554685). An isotype control should be used at comparable concentrations to antibody of interest. For specific methodology, please visit our website protocol section or refer to Chapter 4: Immunofluorescent Staining of Intracellular Molecules for Flow Cytometric Analysis in our handbook: Techniques for Immune Function Analysis Application Handbook 1st Edition. 2003 also found at www.bdbiosciences.com.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
550069	Recombinant Mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)

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

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, Neutralization)

Assenmacher M, Schmitz J, Radbruch A. Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. *Eur J Immunol.* 1994; 24(5):1097-1101. (Clone-specific: Flow cytometry)

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: Flow cytometry)

Sander B, Hoiden I, Andersson U, Moller E, Abrams JS. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. Cytokine detection by immunoassay and intracellular immunostaining. *J Immunol Methods.* 1993; 166(2):201-214. (Clone-specific: Flow cytometry)

Schumacher JH, O'Garra A, Shrader B, et al. The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme-linked immunosorbent. *J Immunol.* 1988; 141(5):1576-1581. (Clone-specific: ELISA, Neutralization)