

Technical Data Sheet

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

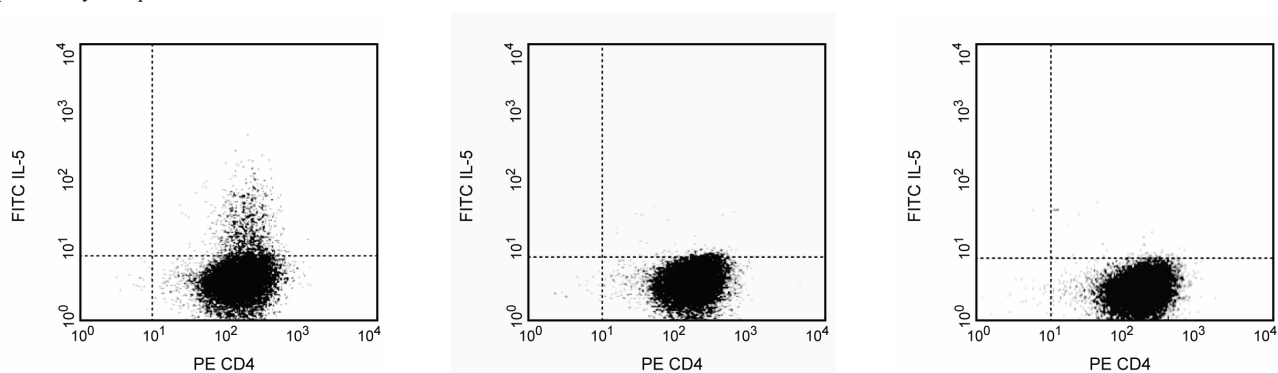
Product Information

Material Number:	554392
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	TRFK5
Immunogen:	Mouse Semi-Purified T-Cell Clone Supernatant
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse, Human
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ 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.

This antibody is routinely tested by ELISA capture. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of IL-5 by stimulated mouse CD4+ T cells. Purified splenic CD4+ cells from BALB/c mice were stimulated with plate-bound anti-CD3 (25 $\mu\text{g/ml}$ final concentration; clone 145-2C11, Cat. No. 553057) and soluble anti-mouse CD28 (2 $\mu\text{g/ml}$ final concentration; Cat. No. 553294) for 2 days in culture together with recombinant mouse IL-2 (10 ng/ml final concentration; Cat. No. 550069) and recombinant mouse IL-4 (1 ng/ml final concentration; Cat. No. 550067), followed by a 3 day incubation with only recombinant mouse IL-2 and IL-4. This was followed by a 5 hour stimulation with plate-bound anti-CD3 (25 $\mu\text{g/ml}$ final concentration) and soluble anti-mouse CD28 (2 $\mu\text{g/ml}$ final concentration) in the presence of GolgiStop™ (2 μM final concentration; Cat. No. 554724). The cells were stained with 0.06 μg of PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553049), fixed, permeabilized, and subsequently stained with 0.12 μg of FITC-conjugated rat anti-mouse IL-5 antibody (FITC-TRFK5, Cat. No. 554394) by using the BD Pharmingen™ staining protocol (left panel). To demonstrate specificity of staining, the binding of FITC-TRFK5 antibody was blocked by the preincubation of the conjugated antibody with recombinant mouse IL-5 (0.1 μg ; Cat. No. 554581; see middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled TRFK5 antibody (5 μg , Cat. No. 554392, see right panel) prior to staining with the FITC-TRFK5 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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

Application Notes

Application

ELISA Capture	Routinely Tested
Blocking	Tested During Development
Western blot	Reported

Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified TRFK5 antibody can be used as a blocking control to demonstrate specificity of IL-5 staining by PE-, FITC-, and APC-TRFK5 antibodies (Cat. No. 554394, 554395, 554396). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1-10 μg of unlabeled TRFK5 antibody (Cat. No. 554392) for 20 minutes at 4°C, prior to staining with PE-, FITC-, or APC-TRFK5 antibody.

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Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
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)
554393	Purified Rat Anti-Mouse/Anti-Human IL-5	0.5 mg	TRFK5

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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.

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, 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)

Mo XY, Sarawar SR, Doherty PC. Induction of cytokines in mice with parainfluenza pneumonia. *J Virol*. 1995; 69(2):1288-1291. (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)

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)

Sarawar SR, Sangster M, Coffman RL, Doherty PC. Administration of anti-IFN-gamma antibody to beta 2-microglobulin-deficient mice delays influenza virus clearance but does not switch the response to a T helper cell 2 phenotype. *J Immunol*. 1994; 153(3):1246-1253. (Clone-specific: ELISA)

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: Neutralization)

Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol*. 1990; 145(11):3796-3806. (Clone-specific: Neutralization)