# **Technical Data Sheet**

# Purified Rat Anti-Mouse IL-4

#### **Product Information**

 Material Number:
 554433

 Size:
 0.1 mg

 Concentration:
 0.5 mg/ml

 Clone:
 11B11

Immunogen: Partially Purified Mouse IL-4

Isotype: Rat IgG1

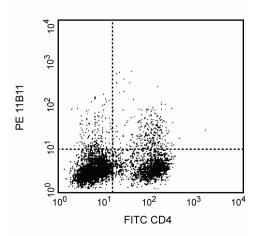
**Reactivity:** QC Testing: Mouse

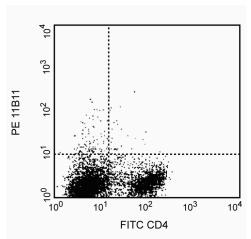
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

#### Description

The 11B11 antibody reacts with mouse interleukin-4 (IL-4). The immunogen used to generate the 11B11 hybridoma was partially purified mouse IL-4 from PMA-stimulated EL-4 supernatant. This is a neutralizing antibody.

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





Expression of IL-4 by stimulated CD4+ and CD4-BALB/c spleen cells. Splenocytes from 6 month old BALB/c mice were cultured for 72 hours in medium containing Staphylococcus aureus enterotoxin B (2 μg/ml final concentration; Sigma, St. Louis, MO), recombinant mouse IL-2 (10 ng/ml final concentration; Cat. No. 550069) and recombinant mouse IL-4 (2 ng/ml final concentration; Cat. No. 550069). The cells were harvested and restimulated for 5 hours with anti-CD3 (2 μg/ml final concentration; 145-2C11, Cat. No. 553057) and anti-CD28 (2 μg/ml final concentration; clone 37.51, Cat. No. 553294) antibodies in the presence of GolgiStop™ (2 μg final concentration; Cat. No. 554704). The splenocytes were harvested, stained with 0.06 μg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.25 μg of PE-conjugated rat anti-mouse IL-4 antibody (PE-11B11, Cat. No. 554435) by using Pharmingen's staining protocol (see left panel). To demonstrate specificity of staining, the binding of PE-11B11 was blocked by the preincubation of the conjugated antibody with recombinant mouse IL-4 (0.25 μg, Cat. No. 550067; data not shown), and by preincubation of the fixed/permeabilized cells with unlabelled 11B11 antibody (5 μg, Cat. No. 554433; see right panel). 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.

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#### **Application Notes**

#### Application

ELISA	Routinely Tested	
Blocking	Tested During Development	
Western blot	Reported	

#### Recommended Assay Procedure:

1. ELISA: The purified 11B11 antibody (Cat. No. 554434) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-4 protein levels. Purified 11B11 antibody can be paired with the biotinylated BVD6-24G2 antibody (Cat. No. 554390) as the detection antibody, with recombinant mouse IL-4 (Cat. No. 550067) as the standard. See the ELISA protocol section in this catalog for specific methodology. These reagents are recommended primarily for measuring cytokine from cell culture systems and are not recommended for assay of serum or plasma samples. For testing IL-4 in serum or plasma, our BD OptEIA<sup>TM</sup> mouse IL-4 set (Cat. No. 555232) is recommended.

2. Neutralization: The NA/LETM 11B11 antibody (Cat. No. 554432) is useful for neutralization of mouse IL-4 bioactivity.

### **Suggested Companion Products**

Catalog Number	Name	Size	Clone
550069	Recombinant mouse IL-2	20 μg	(none)
550067	Recombinant Mouse IL-4	10 μg	(none)
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
554435	PE Rat Anti-Mouse IL-4	0.1 mg	11B11
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(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.
- 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)

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)

Haak-Frendscho M, Brown JF, lizawa Y, Wagner RD, Czuprynski CJ. Administration of anti-IL-4 monoclonal antibody 11B11 increases the resistance of mice to Listeria monocytogenes infection. *J Immunol.* 1992; 148(12):3978-3985.(Clone-specific: Neutralization)

Lindqvist C, Lundstrom H, Oker-Blom C, Akerman KE. Enhanced IL-4-mediated D10.G4.1 proliferation with suboptimal concentrations of anti-IL-4 receptor monoclonal antibodies. *J Immunol.* 1993; 150(2):394-398.(Clone-specific: Neutralization)

Ohara J, Paul WE. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature*. 1985; 315(6017):333-336. (Immunogen)

Openshaw P, Murphy EE, Hosken NA, et al. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J Exp Med.* 1995; 182(5):1357-1367.(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: Blocking, Flow cytometry)

Sadick MD, Heinzel FP, Holaday BJ, Pu RT, Dawkins RS, Locksley RM. Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon gamma-independent mechanism. *J Exp Med.* 1990; 171(1):115-127.(Clone-specific: Neutralization)

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: ELISA, Flow cytometry) 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)

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