Technical Data Sheet

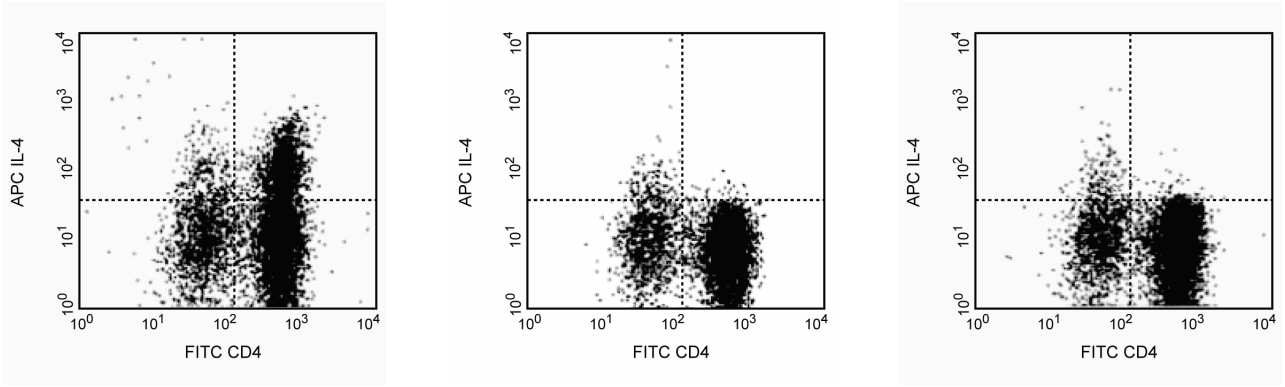
APC Rat Anti-Mouse IL-4

Product Information

Material Number:	**562045**
Size:	25 µg
Concentration:	0.2 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. The purified or unconjugated form of this antibody has been reported to be neutralizing.



Expression of IL-4 by stimulated CD4+ and CD4-Balb/c spleen cells. *BALB/c spleen cells 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 U/ml final concentration; Cat. No. 550069) and recombinant mouse IL-4 (2 ng/ml final concentration; Cat. No. 550067). 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™ (3 µM final concentration; Cat. No. 554724). The splenocytes were then stained with 0.25 µg of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047) and 0.25 µg of APC-conjugated rat anti-mouse IL-4 antibody (APC-11B11, Cat. No. 554436) by using Pharmingen's staining protocol (left panel). To demonstrate staining specificity, the binding of APC-11B11 was blocked by the preincubation of the conjugated antibody with excess recombinant mouse IL-4 (0.25 µg; Cat. No. 550037) (middle panel) or by pre-blocking fixed/permeabilized cells with excess purified "cold" 11B11 mouse antibody (5.0 µg; Cat. No. 554433) (right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified using the cytokine-blocking and "cold" mouse antibody blocking controls. This APC-conjugated reagent can be used in any flow cytometer equipped with a a dye, HeNE or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.*

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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The APC- conjugated 11B11 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-4 producing cells within mixed cell populations. The use of an isotype control, such as rat IgG1 isotype control, APC-R3-34 (Cat. 554686) is recommended. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the Technical Resources/protocols section or the chapter on intracellular staining in the Immune Function Handbook.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
550067	Recombinant Mouse IL-4	10 µg	(none)
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
550069	Recombinant Mouse IL-2	20 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
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554653	MiCK-2 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
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.
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. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
6. An isotype control should be used at the same concentration as the antibody of interest.

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, Iizawa 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)

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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)

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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)