Technical Data Sheet

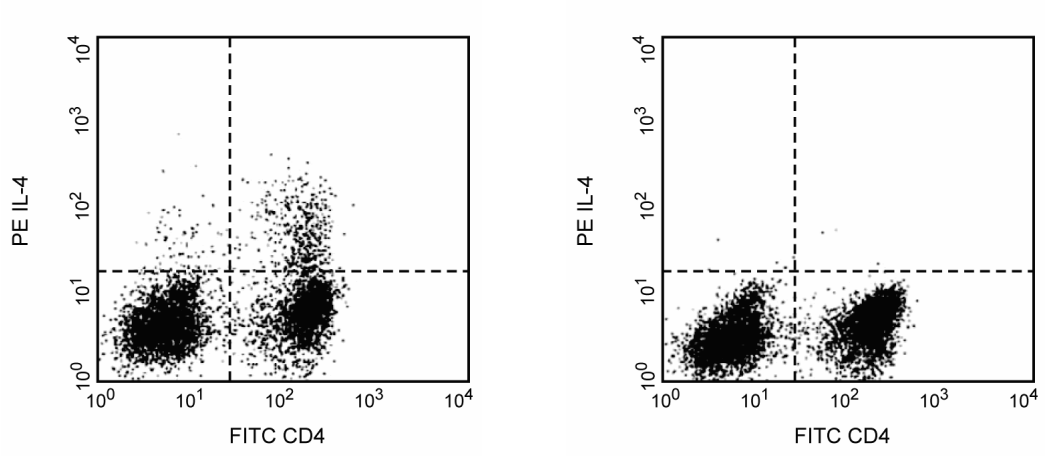
PE Rat Anti-Mouse IL-4

Product Information

Material Number:	**554435**
Size:	0.1 mg
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 h in medium containing Staphylococcus aureus enterotoxin B (2 µg/ml; Sigma, St. Louis, MO), recombinant mouse IL-2 (10 U/ml, Cat. No. 550069) and recombinant mouse IL-4 (2 ng/ml, Cat. No. 550067). The cells were harvested and restimulated for 5 h with anti-CD3 (145-2C11, Cat. No. 553057 at 2 µg/ml) and anti-CD28 (clone 37.51, Cat. No. 553294 at 2 µg/ml) antibodies in the presence of 3 µM monensin (BD GolgiStop, Cat No. 554704). 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 PE-conjugated rat anti-mouse IL-4 antibody (PE11B11, Cat. No 554435) by using the Pharmingen staining protocol (left panel). To demonstrate staining specificity, the binding of PE-11B11 was blocked by the preincubation of the conjugated antibody with excess recombinant mouse IL-4 (0.25 µg; Cat. No. 550067) (right panel) or by pre-blocking fixed/permeabilized cells with excess purified 11B11 mAb (5.0 µg; Cat. No. 554433) (data not shown), prior to staining. An appropriate isotype control is PE-conjugated R3-34, (Cat. No. 554685). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified using the cytokine-blocking or mAb blocking controls.*

Preparation and Storage

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.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

1. Flow Cytometry: The 11B11 antibody has been reported to be useful for the immunofluorescent staining and flow cytometric analysis of IL-4-producing cells. The PE-conjugated 11B11 antibody can be used for multicolor flow cytometric analyses to identify and enumerate IL-4 producing cells within mixed cell populations (see right image). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be pretitrated (≤ 0.5 µg mAb/million cells). An appropriate isotype control is PE-conjugated R3-34, (Cat. No.

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554685). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
550069	Recombinant Mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554653	MiCK-2 Mouse Cytokine Positive Control Cells	1.0 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. 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.

References

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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)

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)

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)

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