Technical Data Sheet

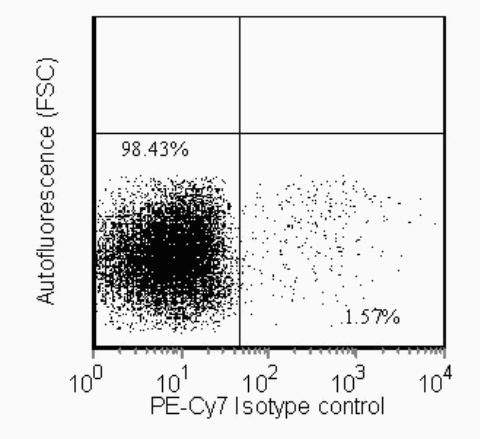
PE-Cy™7 Rat Anti-Mouse IL-4

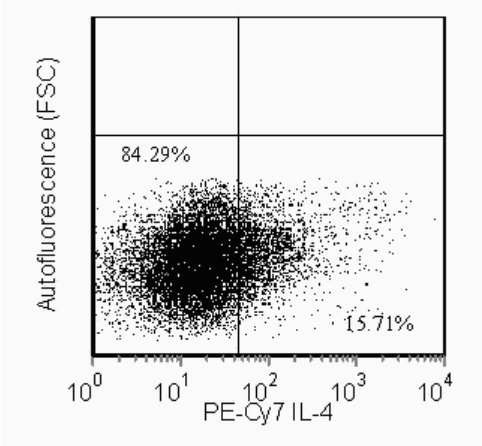
Product Information

Material Number:	**560699**
Size:	50 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.





Flow cytometric analysis for IL-4 in activated mouse splenocytes. *Mouse Intracellular Cytokine-2 positive control cells (MiCK-2) offered by BD Biosciences as MN 554653, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-2 cells were stained either with a PE-Cy™7 Rat IgG1, κ isotype control (left panel) or with the PE-Cy™7 Rat Anti-Mouse IL-4 antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD LSR™ II flow cytometry system.*

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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The 11B11 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-4 producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant mouse IL-4 (Cat. No. 550067) or (2) unlabeled 11B11 antibody (Cat. No. 554434), prior to staining.

Cell Preparation: Investigators not wishing to utilize MiCK-2 cells may alternatively stimulate mouse splenocyte enriched CD4+ cells (e.g C57BL/6) with 10 µg/ml plate-bound NA/LE hamster anti-mouse CD3e antibody (clone 145-2C11; Cat. No. 553057) and 2 µg/ml soluble NA/LE hamster anti-mouse CD28 (clone 37.51; Cat. No. 553294) antibody in the presence of 10 ng/ml recombinant mouse IL-2 (Cat. No. 550069) and 20 ng/ml recombinant mouse IL-4 (Cat. No. 550067) for 2 days followed by additional cell expansion with recombinant IL-2 and IL-4 for an additional 3 days. Following expansion, cells may be activated with the Leukocyte Activation Cocktail (Cat. No. 550583) or alternatively, with a 4-6 hr treatment with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng/mL, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Suggested Companion Products

Catalog Number	Name	Size	Clone
557645	PE-Cy™7 Rat IgG1 κ Isotype Control	0.1 mg	R3-34
554653	MiCK-2 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554434	Purified Rat Anti-Mouse IL-4	0.5 mg	11B11
550067	Recombinant Mouse IL-4	10 µg	(none)
550583	Leukocyte Activation Cocktail, with BD GolgiPlug™	200 µl	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
4. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
7. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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. (Biology)

Bogen SA, Fogelman I, Abbas AK. Analysis of IL-2, IL-4, and IFN-gamma-producing cells in situ during immune responses to protein antigens. *J Immunol.* 1993; 150(10):4197-4205. (Biology)

Chatelain R, Varkila K, Coffman RL. IL-4 induces a Th2 response in Leishmania major-infected mice. *J Immunol.* 1992; 148(4):1182-1187. (Biology)

Finkelman FD, Madden KB, Morris SC, et al. Anti-cytokine antibodies as carrier proteins. Prolongation of in vivo effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J Immunol.* 1993; 151(3):1235-1244. (Biology)

Fujihashi K, McGhee JR, Beagley KW, et al. Cytokine-specific ELISPOT assay. Single cell analysis of IL-2, IL-4 and IL-6 producing cells. *J Immunol Methods.* 1993; 160(2):181-189. (Biology)

Gillis S, Ferm MM, Ou W, Smith KA. T cell growth factor: parameters of production and a quantitative microassay for activity. *J Immunol.* 197; 120(6):2027-2032. (Biology)

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. (Biology)

Helms T, Boehm BO, Asaad RJ, Trezza RP, Lehmann PV, Tary-Lehmann M. Direct visualization of cytokine-producing recall antigen-specific CD4 memory T cells in healthy individuals and HIV patients. *J Immunol.* 2000; 164(7):3723-3732. (Biology)

Klinman D and Nutman T. ELISPOT assay to detect cytokine-secreting murine and human cells. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology.* 1994:6-19. (Biology)

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. (Biology)

Litton MJ, Sander B, Murphy E, O'Garra A, Abrams JS. Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B. *J Immunol Methods.* 1994; 175(1):47-58. (Biology)

Paul WE. Interleukin-4: a prototypic immunoregulatory lymphokine. *Blood.* 1991; 77(9):1859-1870. (Biology)

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)

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. (Biology)

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. (Biology)

Shirai A, Sierra V, Kelly CI, Klinman DM. Individual cells simultaneously produce both IL-4 and IL-6 in vivo. *Cytokine.* 1994; 6(3):329-336. (Biology)

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. (Biology)