Technical Data Sheet

Alexa Fluor® 647 Rat Anti-Mouse IL-4

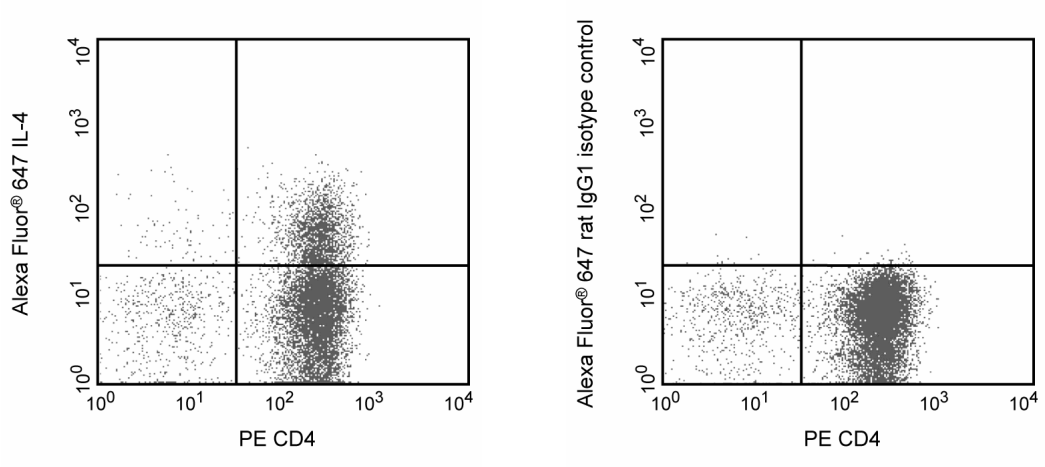
Product Information

Material Number:	**557739**
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. This is a neutralizing antibody.

This antibody is routinely tested by flow cytometric analysis. 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-C57BL/6 spleen cells. *Splenocytes from C57BL/6 mice were enriched for CD4+ cells by positive selection using anti-CD4 coated plates (GK1.5,10 µg/ml, Cat. No.553726) for 1 hr at 4°C. Cells were harvested and stimulated with plate-bound anti-mouse CD3 (145-2C11,10 µg/ml, Cat. No. 553057) and soluble anti-CD28 (37.51,2 µg/ml, Cat. No. 553294) antibody in the presence of recombinant mouse IL-2 (10 ng/ml, Cat. No. 550069) and IL-4 (50 ng/ml, Cat. No. 550067) for 2 days. The cells were subsequently washed and expanded in IL-2 and IL-4 for 3 days. Following expansion the cells were washed and stimulated for 4 hrs with PMA (5 ng/ml, Sigma, Cat. No. P-8139) and ionomycin (500 ng, Sigma, P-8139) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Following incubation the cells were harvested and stained with PE-anti-mouse CD4 (Cat. No. 553048) and either rat anti-mouse IL-4 (Alexa Fluor® 647-11B11, Cat. No. 557739) (left panel) or immunoglobulin isotype control (Alexa Fluor® 647-R3-34, Cat. No. 557731) (right panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 647 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse IL 4 (0.25 µg, Cat. No. 550067, data not shown) and by preincubation of the fixed/permeabilized cells with an excess of unlabelled 11B11 antibody (5 µg, Cat. No. 554433, data not shown) prior to stainining. The quadarant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was 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

BD Biosciences

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877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

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The conjugated 11B11 antibody can be used for multicolor flow cytometric analyses to identify and enumerate IL-4 producing cells within mixed cell populations. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be pretitrated. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Suggested Companion Products

Catalog Number	Name	Size	Clone
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)
557731	Alexa Fluor® 647 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554653	MiCK-2 Mouse Cytokine Positive Control Cells	5x10^6 cells	(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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. 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

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)

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)

Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93.(Clone-specific: ELISA, Flow cytometry)

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, Neutralization)