## **Technical Data Sheet**

# BV421 Rat Anti-Mouse IL-4

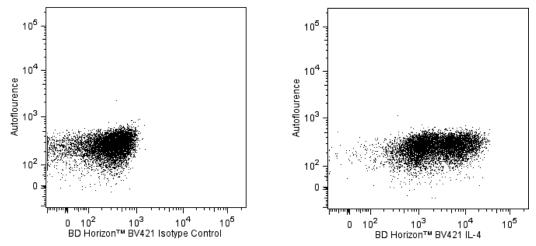
### **Product Information**

Material Number:	562915
Alternate Name:	IL4: Interleukin-4; BSF-1; B-cell growth factor 1; BCGF-1
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 BSA and $\leq 0.09\%$ 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.

The antibody was conjugated to BD Horizon<sup>TM</sup> BV421 which is part of the BD Horizon<sup>TM</sup> Brilliant Violet<sup>TM</sup> family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon<sup>TM</sup> BV421 can be excited by the violet laser and detected in the standard Pacific Blue<sup>TM</sup> filter set (eg, 450/50-nm filter). BD Horizon<sup>TM</sup> BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue<sup>TM</sup> conjugates.



Flow cytometric analysis of IL-4 expressed in activated mouse splenocytes. Splenocytes from C57BL/6 mice were enriched for CD4+ T cells by positive selection using Purified NA/LE Rat Anti-Mouse CD4 antibody-coated plates (GK1.5, Cat. No. 553726;10 µg/ml) for 1 hr at 4°C. The CD4+ T cells were harvested and stimulated with plate-bound Purified NA/LE Hamster Anti-Mouse CD3e (145-2C11, Cat. No. 553057;10 µg/ml) and soluble Purified NA/LE Hamster Anti-Mouse CD28 (37.5). Cat. No. 553294; 2 µg/ml) antibody and Recombinant Mouse IL-2 (Cat. No. 550069; 10 ŋg/ml) and IL-4 (Cat. No. 550067; 50 ŋg/ml) for 2 days. The cells were expanded in IL-2 and IL-4 for 3 days and then washed and stimulated (4 hr) with PMA (Sigma, Cat. No. P-8139; 5 ng/ml) and ionomycin (Sigma, Cat. No. P-8139; 500 ng) in the presence of BD GolgiPlug™ Protein Transport Inhibitor (Containing Brefeldin A) (Cat. No. 555029). The activated cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), permeabilized using the BD Perm/Wash™ Permeabilization Buffer (Cat. No. 554723) and then stained either with a BD Horizon™ BV421 Rat IgG1, κ Isotype Control (Cat. No. 562868, Left Panel) or with BD Horizon™ BV421 Rat Anti-Mouse IL-4 antibody (Cat. No. 562915, Right Panel). Two-color dot plots showing IL-4 (or g isotype control staining) versus autofluorescence were derived from gated events with the forward and light scattering characteristics of intact lymphocytes. Flow cytometry was performed using 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 BD Horizon<sup>TM</sup> BV421 under optimum conditions, and unconjugated antibody and free BD Horizon<sup>TM</sup> BV421 were removed.

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#### **Application Notes**

Application

Intracellular staining (flow cytometry)	Routinely Tested
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#### Suggested Companion Products

Catalog Number	Name	Size	Clone	
562868	BV421 Rat IgG1, κ Isotype Control	50 μg	R3-34	
554656	Stain Buffer (FBS)	500 ml	(none)	
553726	Purified NA/LE Rat Anti-Mouse CD4	0.5 mg	GK1.5	
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)	
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)	
554655	Fixation Buffer	100 ml	(none)	
554723	Perm/Wash Buffer	100 ml	(none)	

#### **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 Brilliant Violet<sup>™</sup> 421 is a trademark of Sirigen.
- 4. Pacific Blue<sup>™</sup> is a trademark of Molecular Probes, Inc., Eugene, OR.
- 5 Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 6. discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 7 For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols. 8.

#### References

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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)

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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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