Technical Data Sheet

V450 Rat Anti-Mouse IL-4

Product Information

Material Number:	560701
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 protein stabilizer and ≤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 is conjugated to BD Horizon[™] V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon[™] V450 can be used in place of Pacific Blue[™] conjugates.



Flow cytometric analysis for IL-4 in activated mouse splenocytes. Mouse Intracellular Cvtokine-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 BD Horizon™ V450 Rat IgG1, κ isotype control (left panel) or with the BD Horizon™ V450 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 BD HorizonTM V450 under optimum conditions, and unreacted BD HorizonTM V450 was 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

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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.

Suggested Companion Products

Catalog Number	Name	Size	Clone	
560535	V450 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	1.0 ml	(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. BD HorizonTM V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
- 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. Pacific Blue[™] is a trademark of Molecular Probes, Inc., Eugene, OR.
- 6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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