

Technical Data Sheet

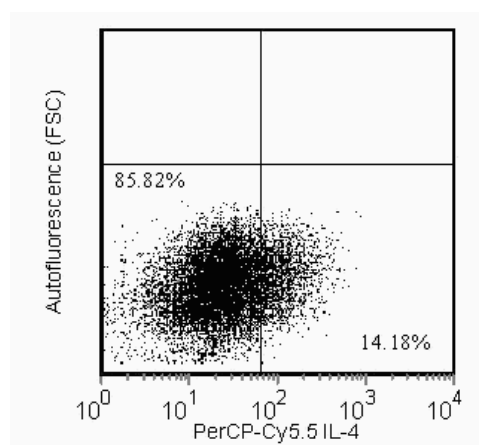
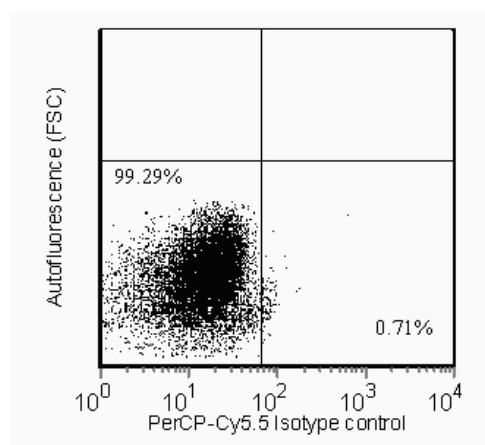
PerCP-Cy™ 5.5 Rat Anti-Mouse IL-4

Product Information

Material Number:	560700
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 PerCP-Cy™ 5.5 Rat IgG1, κ isotype control (left panel) or with the PerCP-Cy™ 5.5 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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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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 CD3ε 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.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
560537	PerCP-Cy5.5 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 μ g	(none)
550583	Leukocyte Activation Cocktail, with BD GolgiPlug™	200 μ l	(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. 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.
4. 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.
5. 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.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. PerCP-Cy5.5-labelled antibodies can be used with FITC- and R-PE-labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
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/pharming/protocols for technical protocols.

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