

Technical Data Sheet

PE-CyTM5 Hamster IgG2, λ 1 Isotype Control**Product Information**

Material Number:	553966
Alternate Name:	Anti-KLH
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	Ha4/8
Immunogen:	Keyhole limpet hemocyanin
Isotype:	Armenian Hamster IgG2, λ 1
Storage Buffer:	Aqueous buffered solution containing \leq 0.09% sodium azide.

Description

The Ha4/8 antibody is specific for the keyhole limpet hemocyanin (KLH).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes**Application**

Flow cytometry	Routinely Tested
Isotype control	Routinely Tested

Recommended Assay Procedure:

This immunoglobulin is useful as an isotype-matched negative control for immunofluorescent staining.

Please observe the following precautions:

PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells.

Furthermore, we have observed a distinct type of interaction between PE-Cy5 tandem fluorochromes and the splenocytes of SJL, NOD and MRL mice: B lymphocytes and some other leukocyte subsets are brightly stained, and Mouse BD Fc Block™ has no significant effect. Therefore, PE-Cy5-conjugated reagents should not be used to stain leukocytes of SJL, NOD, or MRL mice. Reagents conjugated to PE, PerCP, PerCP-Cy5.5, Allophycocyanin (APC), and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
5. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.

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6. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://wwwbdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
7. 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.
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. 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.
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11. An isotype control should be used at the same concentration as the antibody of interest.

References

- Mendrick DL, Kelly DM. Temporal expression of VLA-2 and modulation of its ligand specificity by rat glomerular epithelial cells in vitro. *Lab Invest.* 1993; 69(6):690-702. (Immunogen)
- Takizawa F, Kinet JP, Adamczewski M. Binding of phycoerythrin and its conjugates to murine low affinity receptors for immunoglobulin G. *J Immunol Methods.* 1993; 162(2):269-272. (Biology)
- van Vugt MJ, van den Herik-Oudijk IE, van de Winkle JG. Binding of PE-CY5 conjugates to the human high-affinity receptor for IgG (CD64). *Blood.* 1996; 88(6):2358-2361. (Biology)
- Waggoner AS, Ernst LA, Chen CH, Rechtenwald DJ. PE-CY5. A new fluorescent antibody label for three-color flow cytometry with a single laser. *Ann N Y Acad Sci.* 1993; 677:185-193. (Biology)