Technical Data Sheet

PE-Cy™5 Mouse IgG2a, κ Isotype Control

Product Information

 Material Number:
 553458

 Size:
 0.1 mg

 Concentration:
 0.2 mg/ml

 Clone:
 G155-178

 Immunogen:
 TNP-keyhole limpet hemocyanin

 Isotype:
 Mouse (BALB/c) IgG2a, κ

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The G155-178 clone has an unknown specificity. Trinitrophenol (TNP), the immunogen, is a hapten not expressed on human, mouse, rat or non-human primate cells. In the absence of specific binding, this antibody may bind non-specifically to immunoglobulin Fc receptors. The immunoglobulin secreted by the G155-178 hybridoma was selected as an mouse IgG2a, κ isotype control following screening for low background binding on a variety of mouse and human tissues.

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 PE-Cy5 (formerly known as BD Cy-ChromeTM) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed.

Application Notes

Application

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Flow cytometry	Routinely Tested
Isotype control	Routinely Tested
Intracellular staining (flow cytometry)	Not Recommended

Recommended Assay Procedure:

Note: 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 2.4 G2 (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 splenocytes of SJL, NOD, and MRL mice: B lymphocytes and some other leukocytes subsets are brightly stained, and Mouse BD Fc BlockTM has no significant effect. Therefore, PE-Cy5-conjugated 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

Catalog Number	Name	Size	Clone
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block TM)	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. 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 Cy5TM.
- 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.
- 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.

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 United States
 Canada
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 877.232.8995
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- 7. Cy is a trademark of Amersham Biosciences Limited.
- 8. 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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- 10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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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)

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