Technical Data Sheet

PE-Cy™5 Rat IgG2a, κ Isotype Control

Product Information

Material Number:	**553931**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	R35-95
Immunogen:	Mouse Pooled Immunoglobulin
Isotype:	Rat (LOU) IgG2a, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The R35-95 hybridoma was generated by hybridization of Y3 myeloma cells with spleen cells from LOU rats immunized with mouse immunoglobulins. The R35-95 hybridoma produces rat IgG2a, κ immunoglobulin that has no measurable reactivity with mouse immunoglobulins. The R35-95 immunoglobulin was selected as an isotype control following screening for low background binding on a variety of mouse and human tissues.

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

Recommended Assay Procedure:

An isotype control should be used at the same concentration as the antibody of interest (e.g., ≤ 1 µg/million cells for flow cytometry).

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. Perincubation 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 9APC), and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

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. 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. 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™.
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.

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8. 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.
9. 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 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.
10. 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.

References

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)