Technical Data Sheet

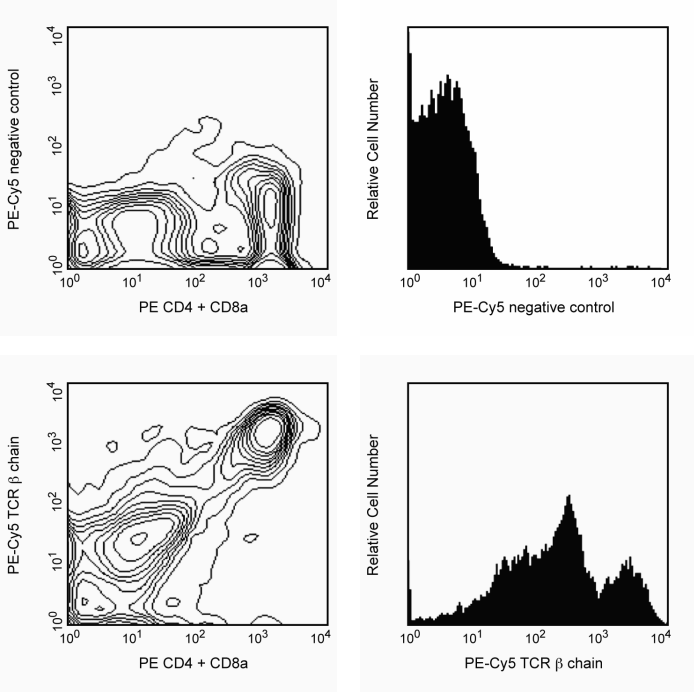
PE-Cy™5 Hamster Anti-Mouse TCR β Chain

Product Information

Material Number:	**553173**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	H57-597
Immunogen:	TCR affinity-purified from mouse T-cell hybridoma DO-11.10
Isotype:	Armenian Hamster IgG2, λ1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The H57-597 antibody reacts with a common epitope of the β chain of the T-cell Receptor (TCR) complex on αβ TCR-expressing thymocytes and peripheral T lymphocytes and NK1.1+ thymocytes and NK-T cells of all mouse strains tested. It does not react with γδ TCR-bearing T cells. In the fetal and adult thymus, the TCR β-chain may form homodimers or pair with the pre-TCR α-chain on the surface of immature thymocytes before expression of the TCR α-chain. Plate-bound or soluble H57-597 antibody activates αβ TCR-bearing T cells, and plate-bound mAb can induce apoptotic death.



αβ TCR expression in spleen and thymus. *BALB/c splenocytes were simultaneously stained with PE-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553048/553049, left panels), PE-conjugated anti-mouse CD8a mAb 53-6.7 (Cat. No. 553032/553033, left panels), and PE-Cy5-conjugated mAb H57-597 (bottom left panel) monoclonal antibodies, in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142, left panels). BALB/c thymocytes were stained with PE-Cy5-conjugated mAb H57-597 (bottom right panel) or unstained (top right panel). Flow cytometry was performed on a BD FACScan™ flow cytometry system.*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

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:

PE-Cy5 tandem fluorochromes have been reported to bind to 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. 553141/5531442) 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

Catalog Number	Name	Size	Clone
553966	PE-Cy™5 Hamster IgG2, λ1 Isotype Control	0.1 mg	Ha4/8

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. 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://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
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.
6. 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.
7. 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.
8. 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.
9. 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

Duke RC, Cohen JJ, Boehme SA, et al. Morphological, biochemical, and flow cytometric assays of apoptosis. In: Coligan J, Kruisbeek AM, Margulies D, Shevach EM, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1995:3.17.1-3.17.33. (Biology)

Gascoigne NR. Transport and secretion of truncated T cell receptor beta-chain occurs in the absence of association with CD3. *J Biol Chem.* 1990; 265(16):9296-9301. (Clone-specific: Immunoprecipitation)

Kruisbeek AM, Shevach EM. Proliferative assays for T cell function. In: Coligan J, Kruisbeek AM, Margulies D, Shevach EM, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1991:3.12.1-3.12.14. (Clone-specific: Stimulation)

Kubo RT, Born W, Kappler JW, Marrack P, Pigeon M. Characterization of a monoclonal antibody which detects all murine alpha beta T cell receptors. *J Immunol.* 1989; 142(8):2736-2742. (Immunogen: Flow cytometry)

Saint-Ruf C, Ungewiss K, Groettrup M, Bruno L, Fehling HJ, von Boehmer H. Analysis and expression of a cloned pre-T cell receptor gene. *Science.* 1994; 266(5188):1208-1212. (Clone-specific: Stimulation)

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. (Clone-specific: Cytotoxicity)