Technical Data Sheet

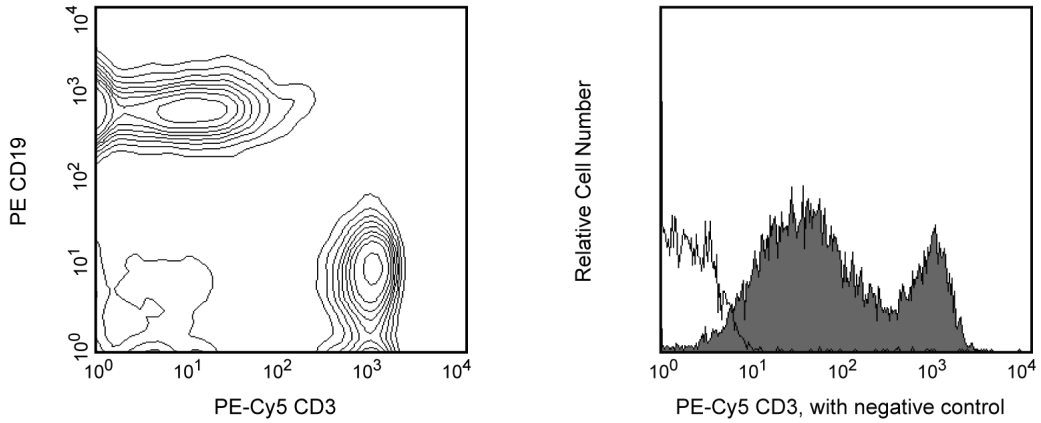
PE-Cy™5 Rat Anti-Mouse CD3 Molecular Complex

Product Information

Material Number:	**555276**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	17A2
Immunogen:	γδ TCR-positive T-T hybridoma D1
Isotype:	Rat (SD) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 17A2 monoclonal antibody specifically binds to the T-cell receptor-associated CD3 complex that is expressed on many thymocytes and mature T lymphocytes. Plate-bound 17A2 antibody has been reported to induce IL-2 production by cultured T cells in the absence of accessory cells. The binding of the 17A2 antibody to T cells can be blocked by the anti-CD3e mAb 145-2C11. This suggests that the 17A2 antibody recognizes an epitope of the CD3 epsilon chain. In vivo treatment with 17A2 antibody has been reported to partially deplete T lymphocytes and temporarily down-modulates CD3 expression on T cells.



CD3 expression in spleen and thymus. *BALB/c splenocytes were preincubated with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) and simultaneously stained with PE-Cy5-conjugated mAb 17A2 and PE-conjugated anti-mouse CD19 mAb 1D3 (Cat. No. 557399/553786, left panel). C3H/HeN thymocytes were stained with PE-Cy5-conjugated 17A2 mAb (right panel, filled histogram) or unstained (right panel, empty histogram). Flow cytometry was performed on a BD FACScan™ flow cytometry system.*

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 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

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mouse: B lymphocytes and some other leukocyte subsets are brightly stained, and Mouse BD Fc Block™ has no significant. 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
553990	PE-Cy™5 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
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. 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.
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. 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.
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.
9. 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.

References

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