# Technical Data Sheet

# APC-Cy™7 Rat Anti-Mouse CD3 Molecular Complex

#### **Product Information**

**Material Number:** 560590 Size: 50 μg 0.2 mg/mlConcentration: 17A2 Clone:

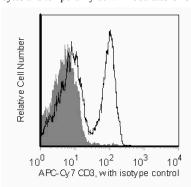
γδ TCR-positive T-T hybridoma D1 Immunogen:

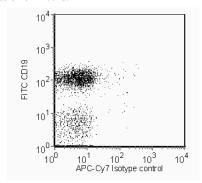
Rat (SD) IgG2b, κ Isotype: Reactivity: QC Testing: Mouse

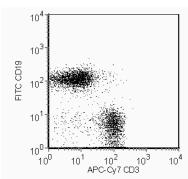
Storage Buffer: Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium

#### 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.







Flow cytometric analysis of CD3 on mouse splenocytes. Left Panel: Splenocytes from C57BL/6 mice were stained with either a APC-Cy™7 Rat IgG2b, κ isotype control (shaded) or with the APC-Cy<sup>™</sup>7 Rat Anti-Mouse CD3 antibody (unshaded). Middle and Right Panels: Splenocytes from C57BL/6 mice were stained with a FITC Rat Anti-Mouse CD19 antibody (Cat.No. 553785) in conjunction with either a APC-Cy™7 Rat IgG2b, κ isotype control (middle panel) or the APC-Cy™7 Rat Anti-Mouse CD3 antibody (right panel). Histograms and dot plots were derived from gated events based on light scattering characteristics for splenocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.

#### 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 APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

## **Application Notes**

### Application

 ppiettion		
Flow cytometry	Routinely Tested	

# **Suggested Companion Products**

Catalog Number	Name	Size	Clone
552773	APC-Cy <sup>TM</sup> 7 Rat IgG2b κ Isotype Control	0.1 mg	A95-1
553785	FITC Rat Anti-Mouse CD19	0.5 mg	1D3

#### **Product Notices**

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- An isotype control should be used at the same concentration as the antibody of interest.
- Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BDTM Stabilizing Fixative (Cat. No. 338036).

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- 4. 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.
- 5. This conjugated product is sold under license to the following patent: US Patent No. 5,714,386.
- 6. 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.
- 7. 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.
- 8. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
- 9. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7<sup>TM</sup>, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
- 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.
- 11. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

Miescher GC, Schreyer M, MacDonald HR. Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. *Immunol Lett.* 1989; 23(2):113-118. (Immunogen)

Mysliwietz J, Thierfelder S. Antilymphocytic antibodies and marrow transplantation. XII. Suppression of graft-versus-host disease by T-cell-modulating and depleting antimouse CD3 antibody is most effective when preinjected in the marrow recipient. *Blood.* 1992; 80(10):2661-2667. (Biology)
Wu L, Antica M, Johnson GR, Scollay R, Shortman K. Developmental potential of the earliest precursor cells from the adult mouse thymus. *J Exp Med.* 1991; 174(6):1617-1627. (Biology)

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