

## Technical Data Sheet

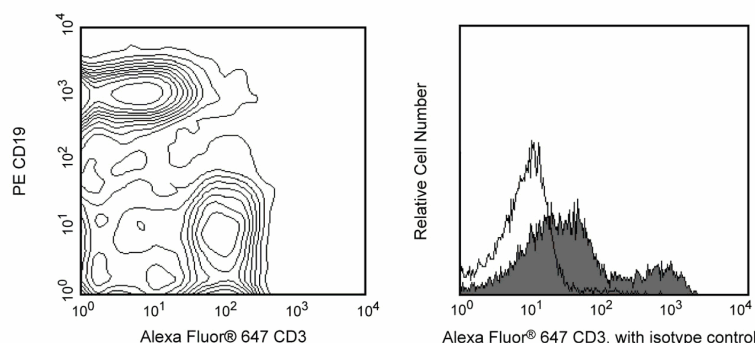
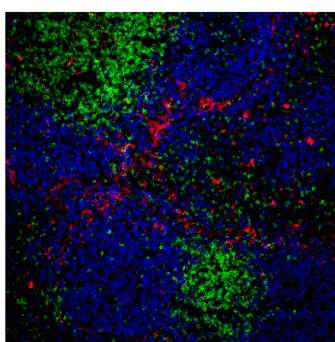
## Alexa Fluor® 647 Rat Anti-Mouse CD3 Molecular Complex

## Product Information

Material Number:	557869
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 (Cat. No. 557306/553058/550275). 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.



**Immunohistofluorescent analysis of CD3 expression by cells within B56BL/6 mouse spleen (left panel).** A mouse spleen cryosection (5 μm) was fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1x PBS, and stained with Alexa Fluor® 647 Rat Anti-Mouse CD3 Molecular Complex antibody (Cat. No. 557869, pseudo-colored green), BD Horizon™ BV421 Rat Anti-Mouse CD11b antibody (Cat. No. 562605, pseudo-colored red), and Alexa Fluor® 488 Rat Anti-Mouse CD45R/B220 antibody (Cat. No. 557669, pseudo-colored blue). Images were captured on a standard epifluorescence microscope. Original magnification, 20x. **CD3 expression in spleen and thymus (middle and right panel).** CD57BL/6 splenocytes were simultaneously stained with Alexa Fluor® 647 mAb 17A2 (Cat. No. 557869) and PE rat anti-mouse CD19 mAb 1D3 (Cat. No. 557399, middle panel). C57BL/6 thymocytes were stained with either Alexa Fluor® 647-conjugated mAb 17A2 (Cat. No. 557869, right panel, filled histogram) or Alexa Fluor® 647-conjugated rat IgG2b, κ isotype control mAb A95-1 (Cat. No. 557691) (right panel, empty histogram). Flow cytometry was performed on a BD FACSCalibur™ 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

## Application Notes

## Application

Flow cytometry	Routinely Tested
Immunofluorescence	Tested During Development

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
557691	Alexa Fluor® 647 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
557399	PE Rat Anti-Mouse CD19	0.1 mg	1D3
554655	Fixation Buffer	100 mL	(none)
562605	BV421 Rat Anti-Mouse CD11b	50 µg	M1/70
557669	Alexa Fluor® 488 Rat Anti-Mouse CD45R	0.1 mg	RA3-6B2
564907	DAPI Solution	1 mg	(none)

### 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. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/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: Cytotoxicity, Functional assay, Immunohistochemistry, Immunoprecipitation, Stimulation)  
Mysliwicz 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. (Clone-specific: Depletion)

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