

Technical Data Sheet

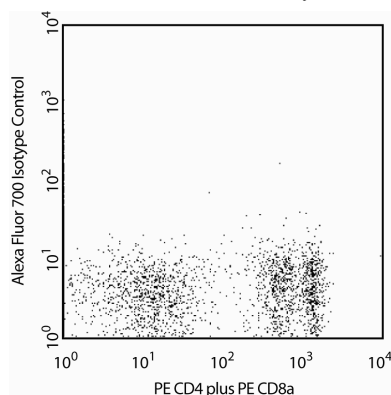
Alexa Fluor® 700 Hamster anti-Mouse CD3e

Product Information

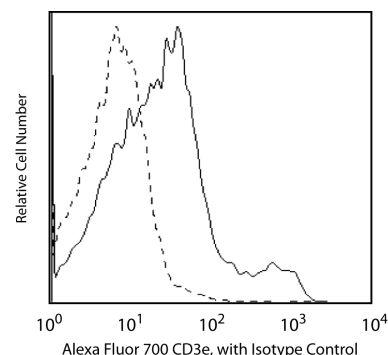
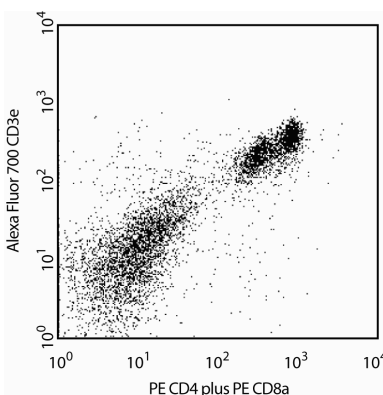
Material Number:	557984
Alternate Name:	CD3ε chain
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	500A2
Immunogen:	Mouse T-cell receptor
Isotype:	Syrian Hamster IgG2, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

This antibody has been reported to react with the 25-kDa ε chain of the T-cell receptor-associated CD3 complex expressed on thymocytes, mature T lymphocytes, and NK-T cells on many mouse stains. Plate-bound and soluble hamster anti-mouse CD3e (clone 500A2) antibody has been reported to activate unprimed T cells in vitro. Activation of a mouse T-cell clone by soluble hamster anti-mouse CD3e (clone 500A2) can be blocked by Fab fragments of anti-CD4 antibody GK1.5, suggesting that the hamster anti-mouse CD3e (clone 500A2) antibody may bind an epitope of CD3e close to a site at which CD4 associates with the T-cell receptor. This hamster mAb to a mouse leukocyte antigen has been reported not to cross-react with rat leukocytes.



CD3e expression in spleen. C57BL/6 splenocytes were simultaneously stained with PE rat anti-mouse CD4 mAb RM4-5 (Cat. no. 553048 or 553049), PE rat anti-mouse CD8a mAb 53-6.7 (Cat. no. 553032 or 553033), and either Alexa Fluor® 700 Hamster IgG2, λ Isotype Control (Cat. no. 557985, left panel) or Alexa Fluor® 700 Hamster anti-Mouse CD3e (right panel). Flow cytometry was performed on a BD™ LSR II flow cytometry system.



CD3e expression in thymus. C57BL/6 thymocytes were stained with either the same isotype control (dashed line) or Alexa Fluor® 700 Hamster anti-Mouse CD3e (solid line). 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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

Note: It has been observed that pre-incubation of thymus cell suspensions at 37°C for 2 to 4 hours prior to staining enhances the ability of anti-CD3e and anti-αβ TCR mAbs to detect the T-cell receptor on immature thymocytes.

Note: Alexa Fluor® 700 conjugates of the hamster IgG2 isotype reportedly can non-specifically stain myeloid cells from mouse bone marrow.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557985	Alexa Fluor® 700 Hamster IgG2, λ Isotype Control	0.1 mg	Ha4/8

BD Biosciences

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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
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. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
8. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Allison JP, Havran WL, Poenie M, et al. Expression and function of CD3 on murine thymocytes. In: Kappler J, Davis M, ed. *The T-Cell Receptor, UCLA Symposia, 73rd Edition*. Los Angeles: 1988:33-45. (Immunogen)

Havran WL, Poenie M, Kimura J, Tsien R, Weiss A, Allison JP. Expression and function of the CD3-antigen receptor on murine CD4+8+ thymocytes. *Nature*. 1987; 330(6144):170-173. (Clone-specific)

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. (Methodology: Flow cytometry)

Ortaldo JR, Winkler-Pickett R, Mason AT, Mason LH. The Ly-49 family: regulation of cytotoxicity and cytokine production in murine CD3+ cells. *J Immunol*. 1998; 160(1):1158-1165. (Clone-specific)

Portoles P, Rojo J, Golby A, et al. Monoclonal antibodies to murine CD3 epsilon define distinct epitopes, one of which may interact with CD4 during T cell activation. *J Immunol*. 1989; 142(12):4169-4175. (Clone-specific)