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Product datasheet for CL001A

Cd3e Hamster Monoclonal Antibody [Clone ID: 145-2C11]

Product data:

Product Type:	Primary Antibodies
Clone Name:	145-2C11
Applications:	FC, FN, IHC, IP, WB
Recommended Dilution:	This antibody will prove useful in studying the role of various components of the TCR complex in T cell activation and development, and will allow for the development of an animal model in which to investigate the immunoregulatory effects of in vivo administration of anti-CD3 antibodies, an area of obvious clinical importance. Anti-CD3 is ideal for flow cytometry applications, particularly as a specific marker for tracking mouse T cells. In addition, this monoclonal antibody, clone 145-2C11 was specifically designed to trigger T cell activation. This clone has also been reported to work in Immunoprecipitation (1, 2) and Western Blotting (Salvadori S. et al. 1994. J. of Immunol. 153: 5176-5182).
Reactivity:	Mouse
Host:	Hamster
Isotype:	IgG
Clonality:	Monoclonal
Immunogen:	H-2K^{b} sp
Specificity:	This monoclonal antibody is specific for a 25 kDa protein component (e-T3) of the antigen specific T cell receptor on all mouse strains tested.
Formulation:	PBS (0.2 µm filtered), with no preservative State: Azide Free State: Liquid purified from ascitic fluid
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Unconjugated
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD3 antigen, epsilon polypeptide

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Database Link: Entrez Gene 12501 Mouse
P22646

Background: The e-T3 protein has been shown to be non-covalently associated on the cell surface alpha/beta heterodimer of the CD3 associated complex. This monoclonal antibody reacts with all mature T cells and can both activate and inhibit T cell function (1). This fact identifies e-T3 as a cell surface protein involved in the transduction of activation signals. All peripheral T cells express this determinant however B cells and bone marrow cells have proven to be negative. Although the expression of this particular epitope on peripheral T cells is uniformly high, staining of thymocytes reveals distinct subpopulations of cells differing in the level of expression of this marker.

Synonyms: T3/Leu-4

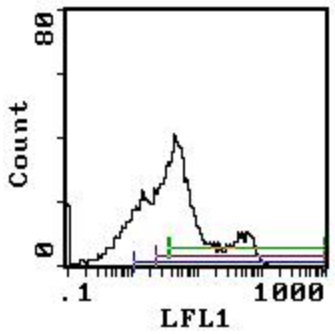
Note: Protocol: **Flow Cytometry analysis**

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration 2x107 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x106 cells, representing one test).
4. To each tube add 0.2 µg of CL001A per 1x106 cells*.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100µl of secondary antibody (f.e. FITC Goat anti-hamster Ig) at a dilution recommended by the manufacturer.
PLEASE NOTE: Do not use PE Goat a hamster IgG as the secondary antibody.
9. Incubate tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in Media B.
11. Resuspend the cell pellet in 50 µl ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in phosphate buffered saline. (This stains dead cells by intercalating DNA).

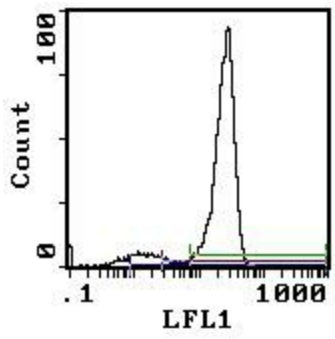
Media: A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2 M sodium azide in 100 mls).

B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls).

Product images:



FLOW CYTOMETRIC ANALYSIS Donor: BALB/c Cell Concentration: 1x10e6 cells Antibody Concentration: 0.5 ug/106 cells Isotypic Control: Purified Hamster IgG Cell Source: A/ Thymocytes, B/ Splenic T Cells Percentage of Cells Stained Above Control A/ 51.5%



B/ 85.7%