

## Product datasheet for **CL001BX**

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

#### Product data:

**Product Type:** Primary Antibodies

**Clone Name:** 145-2C11

**Applications:** FC

**Recommended Dilution:** **Flow Cytometry** (See Protocols).  
This clone has also been reported to work in **Immunoprecipitation**<sup>1,2</sup> and **Western Blotting**<sup>8</sup>.

**Reactivity:** Mouse

**Host:** Hamster

**Isotype:** IgG

**Clonality:** Monoclonal

**Immunogen:** H-2K<sup>b</sup> sp from Armenian Hamster Spleen.

**Fusion Partner:** Murine myeloma cell line SP2/0

**Specificity:** This anti-Mouse CD3e monoclonal antibody is specific for a 25 kDa protein component (e-T3) of the antigen specific T cell receptor on all mouse strains tested. The e-T3 protein has been shown to be non-covalently associated on the cell surface as 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.

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-CD3e 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.



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<b>Formulation:</b>	PBS, 0.2 µm sterile filtered Label: Biotin State: Liquid purified Ascites
<b>Concentration:</b>	lot specific
<b>Purification:</b>	Protein G Affinity Chromatography
<b>Conjugation:</b>	Biotin
<b>Storage:</b>	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
<b>Stability:</b>	Shelf life: one year from despatch.
<b>Gene Name:</b>	CD3 antigen, epsilon polypeptide
<b>Database Link:</b>	<a href="#">Entrez Gene 12501 Mouse P22646</a>
<b>Background:</b>	T cell activation through the antigen receptor (TCR) involves the cytoplasmic tails of the CD3 subunits: CD3 gamma, CD3 delta, CD3 epsilon and CD3 zeta. These CD3 subunits are structurally related members of the immunoglobulins super family encoded by closely linked genes on human chromosome 11. The CD3 components have long cytoplasmic tails that associate with cytoplasmic signal transduction molecules. This association is mediated at least in part by a double tyrosine based motif present in a single copy in the CD3 subunits. CD3 may play a role in TCR induced growth arrest, cell survival and proliferation. The CD3 antigen is present on 68-82% of normal peripheral blood lymphocytes, 65-85% of thymocytes and Purkinje cells in the cerebellum. It is never expressed on B or NK cells. Decreased percentages of T lymphocytes may be observed in some autoimmune diseases.
<b>Synonyms:</b>	T3/Leu-4

**Note:**

Donor: BALB/c

Cell Concentration:  $1 \times 10^6$  cells

Antibody Concentration:  $1 \mu\text{g}/10^6$  cells

Isotypic Control: Biotin Hamster IgG

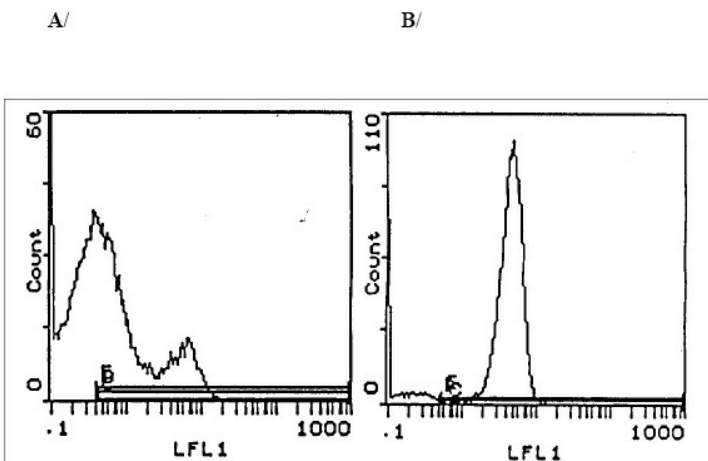
Protocol: **Flow Cytometry Analysis:**

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend cells to  $1 \times 10^6$  cells in approximately 50  $\mu\text{l}$  Media A in a microcentrifuge tube (i.e. 50  $\mu\text{l}$  of cells resuspended to  $2 \times 10^7$  cells/ml). (THE CONTENTS OF 1 TUBE REPRESENTS 1 TEST).
4. To each tube add  $1 \mu\text{g}$  of this antibody per  $10^6$  cells\*.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at  $4^\circ\text{C}$ .
7. Wash 2 times at  $4^\circ\text{C}$ .
8. Add 100  $\mu\text{l}$  of secondary antibody at a 1/500 dilution.
9. Incubate tubes at  $4^\circ\text{C}$  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  $4^\circ\text{C}$  in Media B.
11. Resuspend the cell pellet in 50  $\mu\text{l}$  ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu\text{l}$  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  $\mu\text{l}$  of 2 M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100  $\mu\text{l}$  of 2 M sodium azide in 100 mls).

## Product images:



Cell Source: A/Thymocytes, B/Splenic T Cells  
 Percentage of Cells Stained Above Control:  
 A/50.2, B/87.6