OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CL001

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

Product data:

Product Type:	Primary Antibodies
Clone Name:	145-2C11
Applications:	FC, FN
Recommended Dilution:	Flow Cytometry (see Protocols)
Reactivity:	Mouse
Host:	Hamster
Isotype:	IgG
Clonality:	Monoclonal
Immunogen:	H-2Kb sp. Donor: Armenian Hamster Spleen. Fusion Partner: Murine myeloma cell line SP2/0.
Specificity:	This anti-mouse CD3epsilon monoclonal antibody is specific for a 25 kDa protein component (epsilon-T3) of the antigen specific T cell receptor on all mouse strains tested. The epsilon-T3 protein has been shown to be non-covalently associated with 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 epsilon-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 is useful in studying the role of various components of the TCR complex in T cell activation and development. This antibody 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. In addition, this monoclonal antibody, clone 145-2C11 was specifically designed to trigger T cell activation.
Formulation:	State: Ascites State: Lyophilized Ascites
Reconstitution Method:	Restore with 0.5 ml of cold distilled water.
Concentration:	lot specific

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Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C to -20°C. Following reconstitution store the antibody (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD3 antigen, epsilon polypeptide
Database Link:	Entrez Gene 12501 Mouse P22646
Background:	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.
Synonyms:	T3/Leu-4

Note:

Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:

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 1x10e6 cells in approximately 50 µl Media A in a microcentrifuge tube (ie. 50 µl of cells resuspended to 2x10e7 cells/ml). THE CONTENTS OF 1 TUBE REPRESENTS 1 TEST).
4. To each tube add 50 µl of 1/250-1/500 dilution of CL001.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C. (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat a hamster IgG;) at 1/70 dilution.
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:

CELL SOURCE	PERCENT STAINING
A/ Thymus	59.8
B/ Splenic T Cells*	92.9

*(T cells isolated with CL001)

A. **B.**