

## Monoclonal Antibody to CD3 - FITC

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| <b>Alternate names:</b> | T-cell surface antigen T3/Leu-4, T-cell surface glycoprotein CD3, T3/Leu-4  |
| <b>Catalog No.:</b>     | CL001FX   |
| <b>Quantity:</b>        | 0.3 mg  |
| <b>Concentration:</b>   | 0.1 mg/ml   |
| <b>Background:</b>      | <p>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.</p> |
| <b>Uniprot ID:</b>      | <a href="#">P22646</a>  |
| <b>NCBI:</b>            | <a href="#">NP_031674.1</a>   |
| <b>GeneID:</b>          | <a href="#">12501</a>   |
| <b>Host / Isotype:</b>  | Hamster / IgG   |
| <b>Clone:</b>           | 145-2C11  |
| <b>Immunogen:</b>       | H-2Kb sp from Armenian Hamster Spleen.<br>Fusion Partner: Murine myeloma cell line SP2/0  |
| <b>Format:</b>          | <b>State:</b> Liquid<br><b>Buffer System:</b> PBS + 0.02% NaN <sub>3</sub> and BSA was added to bring final protein concentration to 4-5 mg/ ml.<br><b>Label:</b> FITC  |
| <b>Applications:</b>    | Flow Cytometry (see Protocols)<br>Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.  |

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- Specificity:** This anti-mouse T3 complex 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 with the cell surface 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.  
**Species:** Mouse.  
Other species not tested.
- Add. Information:** **STRAIN DISTRIBUTION:**  
Procedure: as above  
Antibody Concentration: 1µg/10e6 cells  
Strains Tested: BALB/c, C57BL /6, C3H/He, AKR/J, CBA/J  
Positive: BALB/c, C57BL/6, C3H/He, AKR/J, CBA/J
- Storage:** Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.  
Shelf life: one year from despatch.
- General References:** 1. Leo, O. et al. 1987. Proc. Natl Acad. Sci. USA 84: 1374-1378.  
2. Portoles, P. et al. 1989. J. of Immunol. 142: 4168-4175.  
3. Bluestone, J.A. et al. 1987. Nature. 326: 82-84.  
4. Hirsch, R. et al. 1988. J. of Immunol. 140: 3766-3772.  
5. Hirsch, R. et al. 1989 J. of Immunol. 142: 737-743.  
6. Ernst, D.N. et al. 1989. J. of Immunol. 142: 1413-1421.  
7. Flamand, V. et al. 1990. J. of Immunol. 144: 2875-2882.  
8. Salvadori S. et al. 1994. J. of Immunol. 153: 5176-5182.  
9. Denkers, E.Y. et al. 1997. J. of Immunol. 159: 1903-1908.  
10. Brunmark, A. and A.M. O'Rourke. 1997. J. of Immunol. 159: 1676-1685  
11. Lahvis G.P. and J. Cerny. 1997. J. of Immunol. 159: 1783-1793.  
12. Chao, C. et al. 1997. J. of Immunol. 159: 1686-1694.  
13. Chung, C.D. et al. 1997. J. of Immunol. 159: 1758-1766.  
14. Berg, N.N. and H. L. Ostergaard. 1997. J. of Immunol. 159: 1753-1757.
- Protocols:** **FLOW CYTOMETRIC 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 the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing one test).  
4. To each tube add 1.0 - 0.5 µg of this antibody per 1x10e6 cells\*.  
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.

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8. Resuspend the cell pellet in 50  $\mu$ l ice cold Media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ 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  $\mu$ l of 2 M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100  $\mu$ l of 2 M sodium azide in 100 mls).

**FLOW CYTOMETRIC ANALYSIS:**

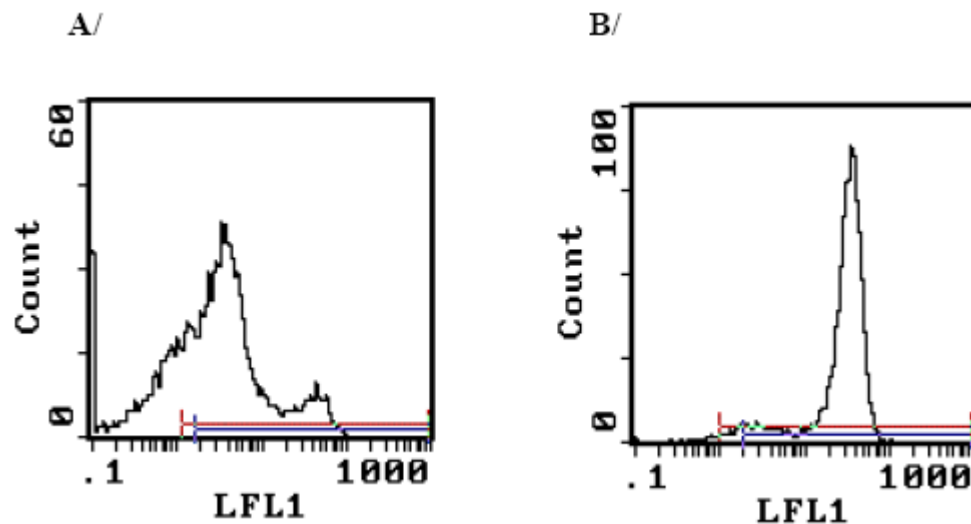
Donor: BALB/c

Cell Concentration: 1x10<sup>6</sup> cells

Antibody Concentration: 0.5  $\mu$ g/10<sup>6</sup> cells

Isotypic Control: FITC Hamster IgG

**Pictures:**



Cell Source: A/Thymocytes, B/Splenic T Cells Percentage of Cells Stained Above Control: A/ 66.8 B/ 94.7

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