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CL004RX **Monoclonal Antibody to CD4 - PE**

Alternate names: T-cell surface antigen T4/Leu-3, T-cell surface glycoprotein CD4

Quantity: 0.3 mg **Concentration:** $0.1 \, \text{mg/ml}$

Background: Mouse CD4 has also been reported to be present on multipotential hematopoietic

> stem cells, bone marrow myeloid precursors, and intrathymic precursors2,3. As a coreceptor in the TCR complex, CD4 is involved in T cell activation through interaction with MHC class II on APC's and in signal transduction via protein tyrosine kinase lck1.

Uniprot ID: P06332

NCBI: NP 038516.1

GeneID: 12504

Host / Isotype: Rat / IgG2a CT-CD4 Clone:

Format: State: Liquid purified IgG

Buffer System: PBS, 0.1% NaN3 and EIA grade BSA as a stabilizing protein to bring

total protein concentration to 4-5 mg/ml

Label: PE

Applications: Flow Cytometry (see Protocols).

> (Reported to be useful in immunohistochemistry on acetone fixed frozen sections.) Other applications not tested. Optimal dilutions are dependent on conditions and

should be determined by the user.

Specificity: This monolconal antibody (mAb) recognizes mouse CD4 (L3T4)

which is expressed on the majority of thymocytes and on the MHC class II restricted

subset of mature T cells including Th cells.

Species: Mouse.

Other species not tested.

Storage: Store the antibody undiluted at 2-8°C.

DO NOT FREEZE!

This antibody is photosensitive and should be protected from light.

Shelf life: one year from despatch.

1. Bierer, B.E., et al. 1989. Annu. Rev. Immunol. 7: 579-599. **General Readings:**

2. Fredrickson, G.G., and R.S. Basch. 1989. J. Exp. Med. 169: 1473-1478.

3. Wu, L., et al. 1991. Nature. 349: 71-74

4. Cobbold, S.P. et al. 1984 Nature. 312: 548-551. 5. Agel, N.M. et al. 1984 J. Immunol. 131: 2445-2451. 6. Dialynas, D.P. et al. 193 J. Immunol. 131: 2445-2451. 7. Palathumpat, V. et al. 1992 J. Immunol. 148: 3319-3326.

8. Gross, J.A. et al. 1992 J. Immunol. 149:380-388. 9. Darby, C.R. et al. 1993 J. Immunol. 159: 125-129. 10. Darby, C.R. et al. 1992 J. Immunol. 54: 483-490.



Protocols:

FLOW CYTOMETRY ANALYSIS:

- 1. Prepare a 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 μ l of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
- 4. To each tube, add \sim 1.0 μ g* of this Ab per 10e6 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 the tubes are protected from light, since most fluorochromes are light sensitive.)
- 7. Wash 2 times at 4°C.
- 8. Resuspend the cell pellet in 50 μl ice cold media B.
- 9. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

MEDIA:

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

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

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

<u>Cell Concentration</u>: 1x10e6 cells per test

Antibody Concentration Used: 1.0 μg/10e6 cells

Isotypic Control: PE Rat IgG2a

Pictures:

Cell Source: Spleen Percentage of cells

stained above control: 23.3%

