

Monoclonal Antibody to CD5 - FITC

Alternate names: CD5, LEU1, Lymphocyte antigen T1/Leu-1, T-cell surface glycoprotein CD5

Catalog No.: CL006FX

Quantity: 0.3 mg

Concentration: 0.1 mg/ml

Background: CD5 is a 55kDa T lymphocyte single chain transmembrane glycoprotein. It is present on all

mature T lymphocytes, on most thymocytes and on many T cell leukemias and lymphomas. It reacts with a subpopulation of activated B cells. CD5/Lyt1 antigen is a monomeric type I transmembrane glycoprotein expressed on thymocytes, T lymphocytes, and a subset of B lymphocytes, but not on natural killer (NK) cells. It has been identified as the major ligand of the B cell antigen CD72. The frequency of CD5+ B cells exhibits strain dependent variation, and the phenotypic, anatomical, functional, developmental, and pathological characteristics of the CD5+ B cells suggest that they may represent a distinct lineage, known as B1 cells. Binding of CD5 on the T cell surface can augment alloantigen or mitogen induced lymphocyte proliferation and induces increased cytosolic free calcium, IL2

secretion, and IL2R expression. It has been proposed that CD5 negatively regulates signal

transduction mediated by the T cell and B cell receptors.

Uniprot ID: P13379

NCBI: NP 031676.3

GenelD: <u>12507</u>

Host / Isotype: Mouse / IgG2b

Clone: CG16

Immunogen: C3H.CE - Ly 1.2 : DS from C3H spleen.

Fusion Partner: Myeloma SP2/0 - Ag 14 (M5).

Format: State: Liquid purified IgG

Purification: Protein G Chromatography

Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total

protein concentration to 4-5 mg/ml

Label: FITC

Applications: Flow cytometry analysis (see Protocols).

Other applications not tested. Optimal dilutions are dependent on conditions and should

be determined by the user.

Specificity: This mAb reacts with T cells from mouse strains expressing the Ly 1.2 phenotype, but does

not react with lymphocytes from mouse strains expressing the Ly 1.1 phenotype.

Species: Mouse.

Other species not tested.



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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.

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:

- 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 $0.1 0.2 \mu g^*$ 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.
- 7. Wash 2 times at 4°C.
- 8. Resuspend the cell pellet in 50 μ l 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).

Results - Tissue Distribution

Mouse Strain: BALB/c

<u>Cell Concentration</u>: 1x10e6 cells per tests <u>Antibody Concentration Used</u>: 0.2 μg/10e6 cells

Isotypic Control: FITC Mouse IgG2b

Results - Strain Distribution

<u>Cell Concentration</u>: 1x10e6 cells per tests <u>Antibody Concentration Used</u>: 0.2 µg/10e6 cells <u>Strains Tested</u>: AKR, ATH, BALB/c, CBA/J, C3H/He

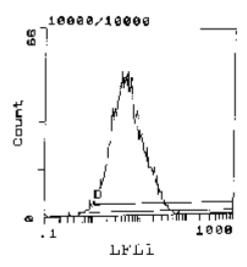
<u>Positive</u>: AKR, ATH, BALB/c <u>Negative</u>: CBA/J, C3H/He

Pictures:

Cell Source	Percentage of cells stamed above control:
Thymus	98.9%
Spleen	33.5%
Lymph Node	88.6%
Bone Marrow	3.3%

FLOW CYTOMETRY ANALYSIS





Cell Source: Thymus Percentage of cells stained above control: 98.9 %FLOW CYTOMETRY ANALYSIS - Tissue Distribution