

Product datasheet for **CL006**

Cd5 Mouse Monoclonal Antibody [Clone ID: CG16]

Product data:

Product Type:	Primary Antibodies
Clone Name:	CG16
Applications:	CT
Recommended Dilution:	Cytotoxicity analysis (see protocol).
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	C3H.CE-Ly 1.2:DS Donor: C3H spleen Fusion Partner: Myeloma SP2/0 - Ag 14 (M5)
Specificity:	This anti-mouse CD5 (Ly 1.2) monoclonal antibody reacts with all T lymphocytes from mouse strains expressing the Ly 1.2 phenotype, but does not react with lymphocytes from mouse strains expressing the Ly 1.1 phenotype.
Formulation:	State: Ascites State: Lyophilized Ascites filtered to 0.45 μ (non-sterile)
Reconstitution Method:	Restore with 1.0 ml of cold distilled water.
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C. Following reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD5 antigen
Database Link:	Entrez Gene 12507 Mouse P13379



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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.

Synonyms: CD5, LEU1

Note: Protocol: **CYTOTOXICITY ANALYSIS:**

Method:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®- Mb density cell separation medium. After washing, adjust the cell concentration to 1×10^6 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:100 and mix.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox®-M Rabbit Complement diluted to the recommended concentration in Cytotoxicity Medium.
6. Incubate for 60 minutes at 37°C.
7. Place on ice.
8. Add Trypan Blue, 10% by volume of 1% Trypan Blue (w/v) added 3-5 minutes before scoring works well. Score live versus dead cells in a hemacytometer.

Results - Antibody Titration by Cytotoxicity Analysis:

Cell Source: Thymus

Donor: BALB/c (Ly 1.2)

Cell Concentration: 1.1×10^6 cells/ml

Complement: Low-Tox®-M Rabbit Complement

Complement Concentration: 1:12

Procedure: Two-stage cytotoxicity as described

Results - Antibody Tissue Distribution by Cytotoxicity Analysis:

Antibody Concentration Used: 1:40

Strain: BALB/c

Results - Strain Distribution by Cytotoxicity Analysis:

Antibody Concentration Used: 1:300

Strains Tested: A.TH, BALB/c, AKR/J, C3H/He, CBA/J

Positive: A.TH, BALB/c, AKR/J

Negative: C3H/He, CBA/J

CYTOTOXICITY DEPLETION ASSAY:

Method:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1×10^7 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:20 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:20 in Cytotoxicity Medium.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox-M® Rabbit Complement, diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox-M® Rabbit Complement)
6. Incubate for 60 minutes at 37°C.
7. Monitor for percent cytotoxicity at this stage, before further processing. For this purpose, remove a small sample from each tube, dilute 1:10 with medium, and add 1/10 volume of 1% Trypan Blue. After 3-5 minutes, score live versus dead cells in a hemacytometer.
8. For functional studies, remove the dead cells from the treated groups before further processing, particularly if the treated cells are to be cultured. This can be done by layering the cell suspension over a Lympholyte®-M and centrifuging at room temperature as per the instructions provided. Live cells will form a layer at the interface, while the dead cells pellet. The interface can then be collected and washed in Cytotoxicity Medium before being resuspended in the appropriate medium for further processing. Alternatively, the cells can be washed and resuspended in the appropriate medium for further processing immediately after Step #6, provided that the dead cells will not interfere with subsequent assays.

FUNCTIONAL TESTING:

Method:

Cells were treated as described in Cytotoxicity Depletion Assay. Treated cells and controls were tested for:

- a) the ability to generate plaque-forming cells (PFC) using a modified Jerne haemolytic plaque assay.
- b) the ability to generate cytotoxic T effector cells using a cytotoxic lymphocyte reaction (CTL) assay. Cells were treated both before and after sensitization in the CTL assay.

Results:

Cell Source: Splenocytes

Donors: C57BL/6 and C3H/He

Cell Concentration: 1×10^7 cells/ml

Antibody Concentration Used: 1:10

Complement: Low-Tox®-M Rabbit Complement

Complement Concentration Used: 1:10

Treatment of C57BL/6 splenocytes with CL8912A plus complement resulted in a significant

reduction in the number of plaque-forming cells. As assessed by a CTL assay, cytotoxic T cell function was essentially eliminated in both presensitized and postsensitized treated samples. No effect in either assay was observed when C3H/He cells were used. These results are consistent with the removal of T helper and T cytotoxic cell activity.

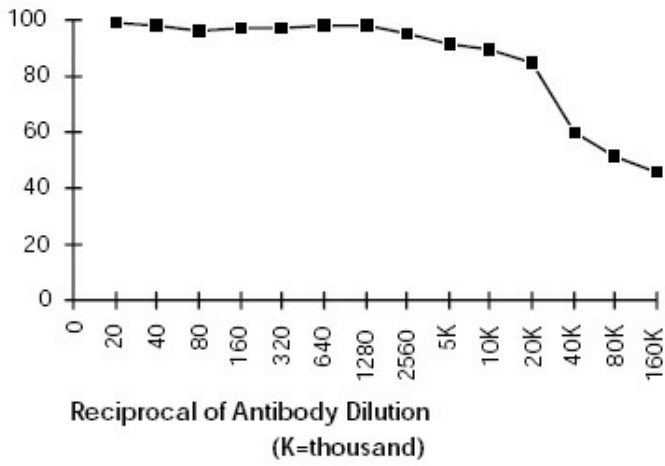
Notes:

- a. Cytotoxicity Medium is RPMI-1640 with 25 mM Hepes buffer and 0.3% bovine serum albumin (BSA). BSA is substituted for the conventionally used fetal calf serum (FCS) because we have found that many batches of FCS contain complement-dependent cytotoxins to mouse lymphocytes, thus increasing the background killing in the presence of complement. Some batches of BSA also contain complement-dependent cytotoxins, resulting in the same problem. We screen for batches of BSA giving low background in the presence of complement and use the selected BSA for preparing Cytotoxicity Medium.
- b. Lympholyte®-M cell separation medium is a density separation medium designed specifically for the isolation of viable mouse lymphocytes. This separation medium provides a high and non-selective recovery of viable mouse lymphocytes, removing red cells and dead cells. The density of this medium is 1.087-1.088. Isolation of mouse lymphocytes on cell separation medium of density 1.077 will result in high and selective loss of lymphocytes and should be avoided.
- c. Rabbit serum provides the most potent source of complement for use with antibodies to mouse cell surface antigens. However, rabbit serum itself is very toxic to mouse lymphocytes. Low-Tox®-M Rabbit Complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

Product images:

$$\text{C.I.} = 100 \times \frac{\% \text{ cyt (antibody + complement)} - \% \text{ cyt (complement alone)}}{100\% - \% \text{ cyt (complement alone)}} \quad \text{Cytotoxic Index}$$

<u>Cell Source</u>	<u>C.I.</u>	
Thymus	98	CYTOTOXICITY ANALYSIS - Cell Source
Spleen	31	
Lymph Node	63	



Antibody Titration by Cytotoxicity Analysis