

Product datasheet for **CL010S**

Cd8a Mouse Monoclonal Antibody [Clone ID: AD4(15)]

Product data:

Product Type: Primary Antibodies

Clone Name: AD4(15)

Applications: CT, FC

Recommended Dilution: **Cytotoxicity assays.**

Results:

Antibody Titration by Cytotoxicity Analysis:

Cell Source: Thymus

Donor: C57BL/6 (Ly-2.2)

Cell Concentration: 1.1×10^6 cells/ml

Complement: Low-Tox®-M Rabbit Complement.

Complement Concentration: 1/18

Flow Cytometry.

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: 1/2500 in 50 μ l / 10^6 cells

Isotypic Control: Mouse IgM.

Reactivity: Mouse

Host: Mouse

Isotype: IgM

Clonality: Monoclonal

Immunogen: C57BL/6

Donor: B6-Ly-2a spleen

Fusion Partner: Myeloma P3/X63-Ag8

Specificity: Anti-Mouse CD8a (Ly 2.2) monoclonal antibody reacts with a sub-population of T-lymphocytes from Mouse strains expressing the Ly-2.2 phenotype but does not react with lymphocytes from strains expressing the Ly-2.1 phenotype.



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Formulation:	State: Supernatant State: Lyophilized Cell Culture Supernatant with no additional preservatives.
Reconstitution Method:	Restore with 1.0 ml of cold distilled water
Conjugation:	Unconjugated
Storage:	Store lyophilized at 2-8°C for 6 months or at -20°C long term. After reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C long term. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD8 antigen, alpha chain
Database Link:	Entrez Gene 12525 Mouse P01731
Synonyms:	CD8 alpha chain, CD8A, MAL
Note:	Protocol: <u>CYTOTOXICITY ANALYSIS:</u> <u>Method:</u> 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 cell separation medium. After washing, adjust the cell concentration to 1x10 ⁶ cells per ml in Cytotoxicity Medium. 2. Add the antibody to a final concentration of 1/1000 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. Cytotoxic Index (C. I.) can be calculated as follows: $\frac{\% \text{ cyt (antibody + complement)} - \% \text{ cyt (complement alone)}}{100\% - \% \text{ cyt (complement alone)}} \times 100 = \text{C.I.}$ <u>CYTOTOXICITY DEPLETION ASSAY:</u> <u>Method:</u> 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 1x10 ⁶ cells per ml in Cytotoxicity Medium. 2. Add the antibody to a final concentration of 1/1000 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1/1000 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 separation medium 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.

FLOW CYTOMETRY ANALYSIS:

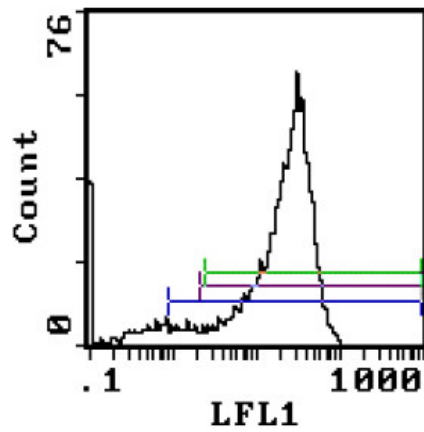
Method:

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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 2.0 μ g of this antibody.
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. Add 100 μ l of secondary antibody (FITC Goat anti-mouse IgM-3 (H+L)) at 1/500 dilution.
9. Incubate the 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 μ l ice cold media B.
12. 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).

Product images:



Cell Source: Thymus. Percentage of cells stained above control: 85.2%

