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Product datasheet for CL010

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

Product data:

Product Type:	Primary Antibodies
Clone Name:	AD4(15)
Applications:	CT, FC
Recommended Dilution:	This clone has been shown to work in both cytotoxicity assays and flow cytometry.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgM
Clonality:	Monoclonal
Immunogen:	Immunogen: C57BL/6 Donor: B6-Ly-2a spleen Fusion Partner: Myeloma P3/X63-Ag8
Specificity:	This anti-mouse CD8a (Ly 2.2) monoclonal antibody reacts with a subpopulation 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.
Formulation:	State: Ascites State: Lyophilized Ascites filtered to 0.45 µm (non-sterile)
Reconstitution Method:	Restore with 0.5 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

View online »

Background: The CD8 antigen is a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell to cell interactions within the immune system. The CD8 antigen, acting as a coreceptor, and the T cell receptor on the T lymphocyte recognize antigen displayed by an antigen presenting cell (APC) in the context of class I MHC molecules. The functional coreceptor is either a homodimer composed of two alpha chains, or a heterodimer composed of one alpha and one beta chain. Both alpha and beta chains share significant homology to immunoglobulin variable light chains.

Synonyms: CD8 alpha chain, CD8A, MAL

Note: Protocol: **CYTOTOXICITY ANALYSIS:**

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-Mb cell separation medium. After washing, adjust the cell concentration to 1x10e6 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 Complementc 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:
Cell Source: Thymus
Donor: C57BL/6 (Ly-2.2)
Cell Concentration: 1.1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:18
Procedure: Two-stage cytotoxicity

Results - Tissue Distribution:
Antibody Concentration Used: 1:1000
Strain: C57BL/6

Results - Strain Distribution:
Antibody Concentration Used: 1:1000
Strains Tested: C57BL/6, CBA/J, BALB/c, AKR/J, ATL, C3H/He
Positive: C57BL/6, BALB/c, ATL
Negative: CBA/J, AKR/J, C3H/He

CYTOTOXICITY DEPLETION ASSAY:

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma 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 1x10e6 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 Complementc, 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 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 50 µl of a 1:2500-1:5000 dilution* of this Ab.
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 µl of secondary antibody (FITC Goat anti-mouse IgM (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 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl 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 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 1:2500 in 50 µl /10e6 cells
Isotypic Control: Mouse IgM

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

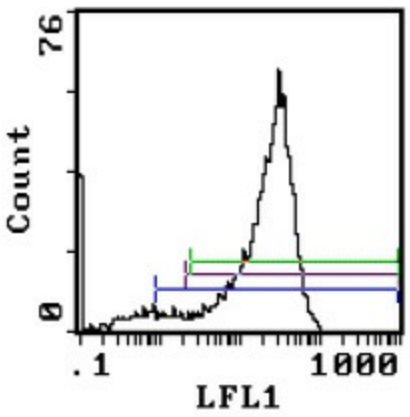
FUNCTIONAL TESTING:

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: BALB/c and C3H/He
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:500
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10
Treatment of BALB/c splenocytes with CL8922A plus complement had no effect on 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. This antibody blocks T cell function in the absence of complement as demonstrated by 51Cr release T-cytotoxicity assay. No effect in either assay was observed when C3H/He cells were used. These results are consistent with the depletion of cytotoxic T cells of the Ly 2.2 phenotype.

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:

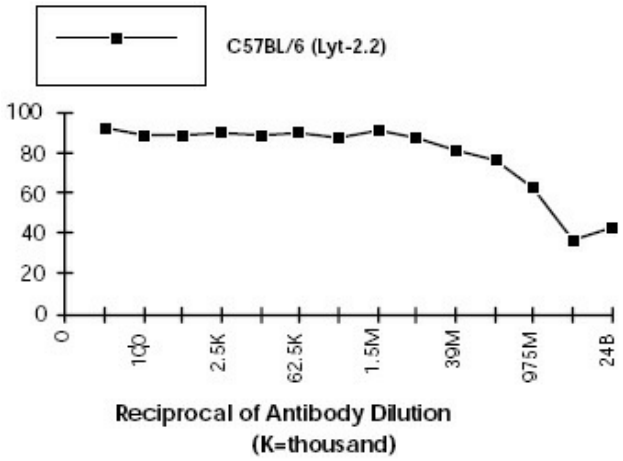


Flow Cytometry Analysis - Cell Source: Thymus - Percentage of cells stained above control: 85.2%

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

Cytotoxic Index

Cell Source	C.I.
Thymus	90
Spleen	9
Lymph Node	13



CYTOTOXICITY ANALYSIS - Antibody Titration