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Product datasheet for CL009S

Cd8a Mouse Monoclonal Antibody [Clone ID: 49-31.1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	49-31.1
Applications:	CT
Recommended Dilution:	Flow Cytometry.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG3
Clonality:	Monoclonal
Immunogen:	Immunogen: CBA Donor: 129/ReJ spleen **Fusion Partner:** Myeloma P3 NSI-Ag 4-1
Specificity:	Anti-mouse Ly-2.1 monoclonal antibody reacts with a sub-population of lymphocytes from mouse strains expressing the Ly 2.1 (CD8a) phenotype, but does not react with lymphocytes from mouse strains expressing the Ly 2.2 phenotype.
Formulation:	PBS containing 0.02% sodium azide (NaN3) as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. State: Supernatant State: Lyophilized tissue culture supernatant with fetal bovine serum.
Reconstitution Method:	Restore with 1,0 ml cold distilled water
Concentration:	lot specific
Purification:	Affinity chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Prior to and following reconstitution store the antibody at 2-8°C for one month or at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD8 antigen, alpha chain

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Database Link: Entrez Gene 12525 Mouse
P01731

Synonyms: CD8 alpha chain, CD8A, MAL

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**
Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium A or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on 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 1/40 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.
Cytotoxic Index (C. I.) can be calculated as follows:
C.I. = (100 x % cyt (antibody + complement) - % cyt (complement alone))/(100% - % cyt (complement alone))
Results:
Antibody Titration by Cytotoxicity Analysis:
Cell Source: Thymus
Donor: C3H/He (Ly 2.1)
Cell Concentration: 1.1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1/18
Procedure: Two-stage cytotoxicity as described
C.I. = (100 x % cyt (antibody + complement) - % cyt (complement alone))/(100% - % cyt (complement alone))
Tissue Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1/40
Strain: C3H/He
Cell Source C.I.
Thymus 96
Spleen 10
Lymph Node 24
Strain Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1/40
Strains Tested: C57BL/6, C3H/He, CBA/J, BALB/c, A.TL, AKR/J
Positive: CBA/J, AKR/J, C3H/He

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Negative: C57BL/6, BALB/c, A.TL

CYTOTOXICITY DEPLETION ASSAY:
Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium A or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on 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 1/40 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1/40 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.

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: 1/10
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1/10
Treatment of C3H/He splenocytes with CL009S plus complement had no effect on the

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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 BALB/c cells were used.
These results are consistent with the depletion of cytotoxic T cells of the Ly 2.1 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.

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