

CL009**Monoclonal Antibody to CD8 - Ascites**

Alternate names:	CD8 alpha chain, CD8A, MAL, T-cell surface glycoprotein CD8 alpha chain, T-lymphocyte differentiation antigen T8/Leu-2
Quantity:	0.5 ml
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.
Uniprot ID:	P01731
NCBI:	NP_001074579.1
GeneID:	12525
Host / Isotype:	Mouse / IgG3
Clone:	49-31.1
Immunogen:	Recipient: 129/ReJ. Donor: CBA. Fusion Partner: Spleen from immunized recipient fused with Myeloma P3 NSI-Ag 4-1.
Format:	State: Lyophilized Ascites Reconstitution: Restore with 0.5 ml of distilled water.
Applications:	Flow Cytometry Analysis. Cytotoxicity Analysis (See Protocols). Functional Testing (See Protocols). Cytotoxicity Depletion Assays (See Protocols). Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This 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. Species: Mouse. Other species not tested.
Add. Information:	Sterility: This reagent is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, dilute to the final working concentration in the appropriate medium before filtration.
Storage:	Prior to and following reconstitution store the antibody at -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.

Protocols:

CYTOTOXICITY ANALYSIS:**Method for Determining percent of Ly 2.1+ cells in a population:**

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 1×10^6 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.

Results - Antibody Titration:

Cell Source: Thymus

Donor: B6-Ly-2a3a (Ly 2.1)

Cell Concentration: 1×10^6 cells/ml

Complement: Low-Tox®-M Rabbit Complement

Complement Concentration: 1:18

Procedure: Two-stage cytotoxicity

Results - Tissue Distribution:

Antibody Concentration Used: 1:500

Strain: B6-Ly-2a3a

Results - Strain Distribution:

Antibody Concentration Used: 1:500

Strains Tested: C57BL/6, C3H/He, B6-Ly-2a3a, CBA/J, BALB/c, DBA/2, A.TH

Positive: B6-Ly-2a3a, CBA/J, DBA/2, C3H/He

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

CYTOTOXICITY DEPLETION ASSAY:**Method for depleting a Ly 2.1+ cell population for further functional analysis:**

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 1×10^6 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:500 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:500 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.

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: C57/BL6 and C3H/He

Cell Concentration: 1x10⁷ cells/ml

Antibody Concentration Used: 1:20

Complement: Low-Tox®-M Rabbit Complement

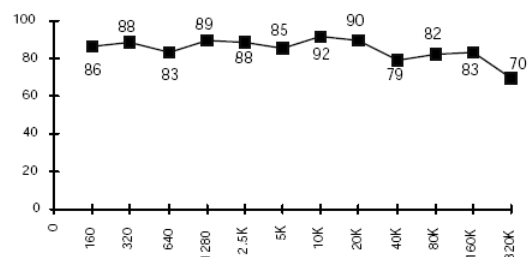
Complement Concentration Used: 1:10

Treatment of C3H/He splenocytes with CL8921A plus complement had no effect on the number of plaque-forming cells. However, marked inhibition of cytotoxic T cell function was found to occur when cells were treated before or after sensitization in the CTL assay. This antibody blocks T cell function in the absence of complement as determined by 51Cr release T-cytotoxicity assay. No effect in either assay was observed when C57BL/6 cells were used. These results are consistent with the depletion of cytotoxic T cells of the Ly 2.1 phenotype.

Pictures:

Antibody Titration by Cytotoxicity Analysis

<u>Cell Source</u>	<u>C.I.</u>
Thymus	88
Spleen	11
Lymph Node	28



Reciprocal of Antiserum Dilution (K= thousand)

STRAIN DISTRIBUTION - Antibody Concentration: 1:500

<u>Strain</u>	<u>Phenotype</u>	<u>+/-</u>
C57BL/6	Ly-2.2	-
CBA/J	Ly-2.1	+
Balb/c	Ly-2.2	-
DBA/2	Ly-2.1	+
C3H/He	Ly-2.1	+
B6-Ly-2 ^{a3}	Ly-2.1	+
A.TH	Ly-2.2	-

Cytotoxic Index (C. I.) can be calculated as follows:

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