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CL011 Monoclonal Antibody to CD8 beta - Ascites

Alternate names: CD8B, CD8B1, T-cell surface glycoprotein CD8 beta chain

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: P10300

NCBI: NP_033988.1

GeneID: 12526

Host / Isotype: Mouse / IgM

Clone: F9(1)

Immunogen: Recipient: ((B6xB6-Ly-2a)F1xB6-Ly-2a)) offspring (Ly-2a homozygous)
Donor: B6-Ly-2a3a,H-2k
Fusion Partner: Spleen from immunized recipient fused with myeloma P3/ X63-Ag8

Format: **State:** Lyophilized Ascites
Reconstitution: Restore with 0.5 ml of cold distilled water.

Applications: Flow Cytometry
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This Anti-Ly-3.1 monoclonal antibody reacts with a sub-population of T-lymphocytes expressing the Ly-3.1 phenotype, but does not react with lymphocytes from mouse strains expressing the Ly-3.2 phenotype.
Species: Mouse.
Other species not tested.

Add. Information: This reagent is not sold 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.

General Readings:
1. Gottlieb, P.D., et al. Construction and Properties of New Lyt-Congenic Strains and Anti-Lyt-2.2 and Anti-Lyt-3.1 Monoclonal Antibodies. Immunogenetics 10:545-555, 1980.
2. Raulet, D.H., et al. Fractionation of Lymphocyte Populations with Monoclonal Antibodies
Specific for Lyt-2.2 and Lyt-3.1 J. Immunol. 125:1136-1143, 1980.

3. Reilly, E.B., et al.. Precipitation with Monoclonal and Conventional Antibodies and Analysis on one and two-dimensional Polyacrylamide Gels. J. Immunol 125:2245- 2251, 1980.

Protocols:

Depleting A Cell Population Of Ly-3.1 Positive Lymphocytes:

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 1x10e7 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:100 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:100 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 vs. 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 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.

Determining Percent of Ly-3.1 Positive Cells in a Population:

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 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 1:100 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 (3). A recommended concentration is included with each batch of complement.
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. Scorelive vs. dead cells in a hemacytometer.

9. Cytotoxic index (C.I.) can be calculated as follows: (see Pictures)

Results - Antibody Titration
Cell Source: Thymus
Donor: C57BL/6 (Ly-3.2) B6-Ly-2a3a (Ly-3.2)
Cell Concentration: 1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:18
Procedure: Two-stage cytotoxicity

Functional Testing:

Method:
Cells were treated as described in Recommended Method for Depleting a Cell Population of Ly-3.1 Positive Lymphocytes. 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. In vitro immunizations were used in all experiments.

Results:
Cell Source: Splenocytes
Donors: C57BL/6 and B6-Ly-2a3a
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:100
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10
Treatment of B6-Ly-2a3a splenocytes with anti-Ly-3.1 plus complement resulted in a significant reduction in the number of plaque-forming cells. However, significant inhibition of cytotoxic T cell functions was found to occur when cells were treated before of after sensitization in the CTL 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-3.1 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 should be avoided, as this will result in high and selective loss of lymphocytes.
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.

Pictures:

Tissue Distribution by Cytotoxicity Analysis

Tissue Distribution by Cytotoxicity Analysis:
Procedure: as above
Antibody Concentration Used: 1:500
Strain: B6-Ly-2^{a}3^{a}

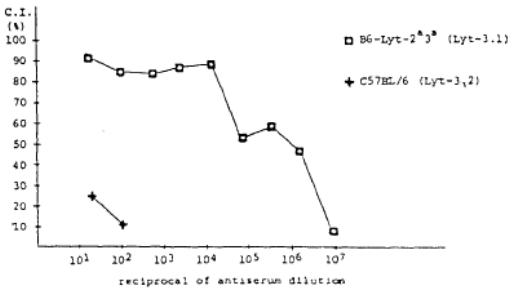
Cell Source	C.I.
Thymus	85
Spleen	12
Lymph Node	29

Strain Distribution by Cytotoxicity Analysis

Strain Distribution by Cytotoxicity Analysis:
Procedure: as above
Antibody Concentration Used: 1:500
Strains Tested:

Strain	Haplotype	+/-
C57BL/6	H-2^{b}	-
CBA/J	H-2^{k}	-
Balb/c	H-2^{d}	-
AKR/J	H-2^{k}	+
B6-Ly-2^{a}3^{a}	H-2^{b}	+
B6-Ly-1a	H-2^{b}	-
C3H/He	H-2^{k}	-
B6-Ly-2a	H-2^{b}	-

Antibody Titration by Cytotoxicity Analysis



Cytotoxic index

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