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# TECHNICALLY *Speaking*

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## **Anti-Ly-3.1 Monoclonal Antibody Ascites CD8b equivalent**

**CL8931A  
LOT: 3101**

### **DESCRIPTION:**

Cedarlane's 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.

This clone has been reported to work in flow cytometry.

### **PRESENTATION:**

0.5 ml, lyophilized.

### **STORAGE/STABILITY:**

Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of distilled water. Aliquot and freeze the unused portion in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

### **STERILITY:**

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.

For more information or to place an order please contact...

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**SPECIFICATIONS:**

Clone: F9(1)

**Hybridoma Production:**

Immunization: Recipient: ((B6xB6-Ly-2<sup>a</sup>)F1xB6-Ly-2<sup>a</sup>) offspring (Ly-2<sup>a</sup> homozygous)  
Donor: B6-Ly-2<sup>a</sup>3<sup>a</sup>,H-2<sup>k</sup>

Fusion Partner: Spleen from immunized recipient fused with myeloma P3/X63-Ag8

Specificity: Ly-3.1

Ig Class: IgM

Presentation: Ascites fluid.

**RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF Ly-3.1 POSITIVE LYMPHOCYTES:****Method:**

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium<sup>®</sup> (CL95100) or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte<sup>®</sup>-M density cell separation medium (CL5030). After washing, adjust the cell concentration to  $1 \times 10^7$  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 Cedarlane Low-Tox<sup>®</sup>-M Rabbit Complement, diluted to the appropriate concentration in Cytotoxicity Medium (CL3051). 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.

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

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium® (CL95100) or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte-M® density separation medium (CL5030). After washing, adjust the cell concentration to  $1 \times 10^6$  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.
3. Centrifuge to pellet the cells and discard the supernatant.
4. Resuspend to the original volume in Cedarlane Low-Tox-M® Rabbit Complement<sup>3</sup> (CL3051). A recommended concentration is included with each batch of complement.
5. Incubate for 60 minutes at 37°C.
6. Place on ice.
7. Add trypan blue. 10% by volume of 1% trypan blue (w/v) added 3-5 minutes before scoring works well. Score live vs. dead cells in a hemacytometer. Cytotoxic index (C.I.) can be calculated as follows:
8. 
$$C.I. = 100 \times \frac{\% \text{ cyt (antibody + complement) - \% cyt (complement alone)}}{100\% - \% \text{ cyt (complement alone)}}$$

Antibody Titration by Cytotoxicity Analysis:

Cell Source: Thymus

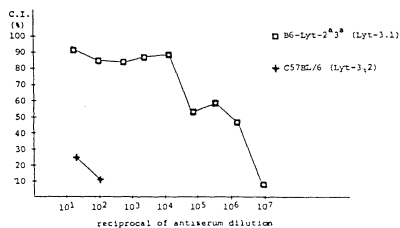
Donor: C57BL/6 (Ly-3.2)

B6-Ly-2<sup>a</sup>3<sup>a</sup> (Ly-3.2)Cell Concentration: 1x10<sup>6</sup> cells/mlComplement: Cedarlane Low-Tox<sup>®</sup>-M Rabbit Complement (CL3051)

Complement Concentration: 1:18

Procedure: Two-stage cytotoxicity as described on page 2.

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

Strain Distribution by Cytotoxicity Analysis:

Procedure: as above

Antibody Concentration Used: 1:500

Strains Tested:

<u>Strain</u>	<u>Haplotype</u>	<u>+/-</u>
C57BL/6	H-2 <sup>b</sup>	-
CBA/J	H-2 <sup>k</sup>	-
Balb/c	H-2 <sup>d</sup>	-
AKR/J	H-2 <sup>k</sup>	+
B6-Ly-2 <sup>a</sup> 3 <sup>a</sup>	H-2 <sup>b</sup>	+
B6-Ly-1a	H-2 <sup>b</sup>	-
C3H/He	H-2 <sup>k</sup>	-
B6-Ly-2a	H-2 <sup>b</sup>	-

Tissue Distribution by Cytotoxicity Analysis:

Procedure: as above

Antibody Concentration Used: 1:500

Strain: B6-Ly-2<sup>a3</sup>

<u>Cell Source</u>	<u>C.I.</u>
Thymus	85
Spleen	12
Lymph Node	29

**FUNCTIONAL TESTING:**

Cell Source: Splenocytes

Donors: C57BL/6 and B6-Ly-2<sup>a3</sup>Cell Concentration: 1x10<sup>7</sup> cells/ml

Antibody Concentration Used: 1:100

Complement: Cedarlane Low-Tox<sup>®</sup>-M Rabbit Complement (CL3051)

Complement Concentration Used: 1:10

Method:

Cells were treated as described in "Recommended Method for Depleting a Cell Population of Ly-3.1 Positive Lymphocytes" on page 2. Treated cells and controls were tested for:

- the ability to generate plaque-forming cells (PFC) using a modified Jerne haemolytic plaque assay.
- 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:

Treatment of B6-Ly-2<sup>a3</sup> 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. Cedarlane Cytotoxicity Medium (CL95100) 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 Cedarlane Cytotoxicity Medium.
- b. Cedarlane Lympholyte<sup>®</sup>-M cell separation medium (CL5030) 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. Cedarlane Low-Tox<sup>®</sup>-M (CL3051) Rabbit Complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cedarlane Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

**REFERENCES:**

1. Gottlieb, P.D., et al. Construction and Properties of New Lyt-Congenetic 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.

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