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Product datasheet for CL048

Ly6a Mouse Monoclonal Antibody [Clone ID: 1147447]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1147447
Applications:	CT
Recommended Dilution:	This antibody is a useful antibody for studies of myeloid differentiation stages and their regulations by cytokines. Applications include flow cytometry (1,2,3) complement mediated depletion (4) Western blot staining (5) and both frozen and paraffin sections (6). Other applications not tested. Optimal dilutions of this antibody are dependent on conditions and should be determined by the user.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Specificity:	The Ly-6 antigens are a complex collection of determinants, probably coded for by a family of closely linked genes, referred to as Ly6A-E. These antigens have been shown to be differentially expressed on thymocytes, peripheral T and B cells, bone marrow cells, macrophages and neutrophils as well as nonhematopoietic tissues. (4) At present, there is little insight into the function of the products of the Ly-6 locus. (1) However, the antigens have been implicated in playing a role in T cell activation. (1) This mouse Ly6A.2 monoclonal antibody detects the classical Ly-6 alloantigen originally defined with conventional antiserum. (2) Ly6A.2 has been shown to be present on 50-70% of peripheral lymphocytes (including CD4+, CD8+ and B cells), on few thymocytes, all B and T cell blasts and is absent from most bone marrow cells and neutrophils. (2) Ly6A.2 has been located in close proximity to Ly6D.2 on the cell surface, and although related to one another, these two determinants are distinct from Ly6C.2 and Ly6B.2. (2) Thus, although the role of Ly6A.2 is unclear, antibodies to this antigen may be useful tools for separating functionally distinct subpopulations of lymphocytes and resolving pathways of differentiation. In fact, Ly6A.2 is absent from any precursor T cells, but is present on effector T cells. Anti-Ly6A.2 antibodies may also prove useful in T cell activation studies as one of the prominent features of the Ly6A.2 specificity is its anhanced expression following T Cell Activation. (1)
Formulation:	State: Ascites

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Conjugation:	Unconjugated
Storage:	Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions in volumes appropriate for single usage. Avoid repeated freezing and thawing. This reagent is not 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 and filter through a 0.45μ Millipore filter (or equivalent).
Stability:	Shelf life: one year from despatch.
Gene Name:	lymphocyte antigen 6 complex, locus A
Database Link:	Entrez Gene 110454 Mouse P05533
Synonyms:	Lymphocyte antigen 6A-2/6E-1, Ly-6A.2/Ly-6E.1, T-cell-activating protein, TAP, Stem cell antigen 1, SCA-1
Note:	CL048/ME1105

Recommended method for depleting a cell population of Ly6A.2 positive lymphocytes:

Method:

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 (CL5030) cell separation medium. After washing, adjust the cell concentration to 1x107 cells per ml in Cytotoxicity Medium.
Add the antibody to a final concentration of 1:20 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:20 in Cytotoxicity Medium.
Incubate for 60 minutes at 4°C.
Centrifuge to pellet the cells and discard the supernatant.
Resuspend to the original volume in Low-Tox®-M Rabbit Complementc (CL3051) diluted to the recommended concentration in Cytotoxicity Medium.
Incubate for 60 minutes at 37°C.
Monitor for present 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 trypan blue. After 3-5 minutes, score live vs, dead cells in a hemacytometer.
For further 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 layering the treated cell suspensions over an equal volume of Lympholyte-M cell separation medium and centrifuging at room temperature as per the instruction 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 resuspended in the appropriate medium for further processing.

Alternatively, the cells can be washed and resuspended in the appropriate medium for

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further processing immediately after Step#6, provided that the dead cells will not interfere with subsequent assays.

Flow Cytometry Analysis of Splenic T Cells:

Donor: C57BL/6
Cell Concentration: 1 x 106 cells per test
Antibody concentration: 1:100
Cell source: Splenic T Cells

Percentage of C57BL/6 cells stained above control - 94.4%
(Mouse T cells isolated with CL101)

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 1x106 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 2 x 107 cells/ml). The content of 1 tube represent 1 test.
4. To each tube, add 50 µl of a 1:50 of CL048. (to make a final dilution of 1/100)
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.

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7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-mouse IgG (H+L)) at a 1:75 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that 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).

Recommended Method for detecting percent of Ly6A.2 positive cells in a population:

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 (CL5030) cell separation medium. After washing, adjust the cell concen-tration to 1x106 cells per ml in Cytotoxicity Medium.
Add the antibody to a final concentration of 1:40 and mix.
Incubate for 60 minutes at 4°C.
Centrifuge to pellet the cells and discard the supernatant.
Resuspend to the original volume in Low-Tox®-M Rabbit Complementc (CL3051) diluted to the recommended concentration in Cytotoxicity Medium.
Incubate for 60 minutes at 37°C.
Place on ice.
Add Trypan Blue, 10% by volume of 1% Trypan Blue (w/v) added 3-5 min-utes 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)

Lot specifications

Lot No. 1406

Antibody Titration

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Cell source: Lymph Node Cells
Donor: C57BL/6
Cell Concentration: 1.1 x 106 cells/ml
Complement: Low-Tox-M Rabbit Complement
Complement concentration: 1:12

Protocol: Two stage cytotoxicity as described above “Recommended method for determination percent of Ly6A.2 positive lymphocytes”.

C.I.= Cytotoxic Index=
100 x % cyt (antibody + complement) - % cyt (complement alone)
100% - % cyt (complement alone)

Strain Distribution

Procedure: as above
Antibody Concentration: 1:20
Strains tested: C57BL/6, ATL, BALB/c, C3H/He, CBA, DBA
Cells killed by treatment: C57BL/6, DBA
Cells not killed by treatment: ATL, BALB/c, C3H/He, CBA

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Tissue Distribution

Procedure: as above
Antibody Concentration: 1:1000
Strain: C57BL/6

Cell Source C.I:
Thymus 3
Spleen 62
Lymph Node 73
Bone Marrow 9

Protein concentration: 51.3 ± 1.8 mg/ml

NOTES:

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 many batches of FCS contain complement-dependent cytotoxins to mouse lymphocytes, thus increasing the background killing in the presence of complement. We recommended that cells not be exposed to FCS prior to or during exposure to antibody and 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.

Lympholyte®-M (CL5030) 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.

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 (CL3051) 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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