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OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CL039S

Thy1 Mouse Monoclonal Antibody [Clone ID: 5a-8]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5a-8
Applications:	CT
Recommended Dilution:	Functional Testing (see Protocols). Cytotoxicity Analysis (see Protocols). Cytotoxicity Depletion Assay (see Protocols).
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	CBA/J. Donor: AKR/J Spleen. Fusion Partner: Myeloma P3 NSI-1-Ag 4-1
Specificity:	This monoclonal antibody reacts with all T lymphocytes from Mouse strains expressing the Thy 1.2 phenotype, (i.e. C57BL/6, C3H/He, DBA/2, CBA/J, BALB/c), but does not react with lymphocytes expressing the Thy 1.1 phenotype [eg. AKR/J, B6.PL (74NS)].
Formulation:	State: Supernatant State: Lyophilized non-sterile Supernatant from Bioreactor Cell Culture
Reconstitution Method:	Restore with 1.0 ml of cold distilled water.
Conjugation:	Unconjugated
Storage:	Prior to and following reconstitution store the antibody at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	thymus cell antigen 1, theta
Database Link:	Entrez Gene 21838 Mouse P01831

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Background: CD90 / Thy1 antigen is a GPI linked glycoprotein member of the Immunoglobulin superfamily. It is expressed on murine T cells, thymocytes, neural cells, cells of granulocytic lineage, early hematopoietic progenitors, fibroblasts, neurons and Kupffer's cells. Thy1 may play a role in cell to cell or cell to ligand interactions during synaptogenesis and other events in the brain. It is found in most mouse strains except AKR/J, A, Thy1.1 and B6.PL (74NS) expressing Thy1.1.

Synonyms: Thy-1, THY1, CDw90

Note: Protocol: **CYTOTOXICITY 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 Lympholyte®-M[b] 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 1:1000 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[c] 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.
9. Cytotoxic Index (C. I.) See Picture.

Results:

Antibody Titration by Cytotoxicity Analysis:
Cell Source: Thymus.
Donor: BALB/c.
Cell Concentration: 1.1x10e6 cells/ml.
Complement: Low-Tox®-M Rabbit Complement.
Complement Concentration: 1:22
Procedure: Two-stage cytotoxicity (See Picture).

Tissue Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:40
Strain: BALB/c

Cell Source C.I.
Thymus: 100
Spleen: 23
Lymph Node: 69

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Bone Marrow: 3

Strain Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:20
Strains Tested: AKR/J, C57BL/6, BALB/c, C3H/He, ATH,CBA/J
Positive: C57BL/6, BALB/c, C3H/He, ATH, CBA/J
Negative: AKR/J

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 Lympholyte®-M 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 1:20 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:20 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 (c), 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:

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Cell Source: Splenocytes
Donors: C57BL/6 and AKR/J
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:20
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10

Treatment of C57BL/6 splenocytes with CL039S plus complement resulted in a significant reduction in the 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 AKR/J cells were used. These results are consistent with the removal of T helper and T cytotoxic cell activity.

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.

Product images:

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

Cytotoxicity Analysis - Cytotoxic Index (C. I.)

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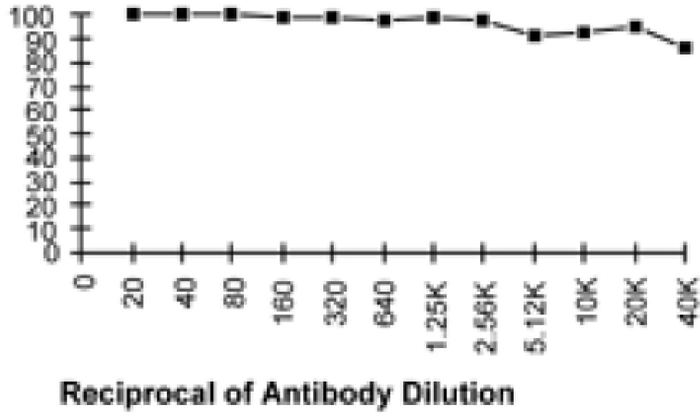

Cytotoxicity Analysis

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