US office:
Acris Antibodies, Inc.
San Diego, CA
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to CD90 - Ascites

Alternate names: CDw90, THY1, Thy-1, Thy-1 membrane glycoprotein

Catalog No.: CL039

Quantity: 0.5 ml

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.

Uniprot ID: P01831

NCBI: NP_033408.1

GeneID: 21838

Host / Isotype: Mouse / IgG2b

Clone: 5a-8

Immunogen: CBA/J
Donor: AKR/J Spleen
Fusion Partner: Myeloma P3 NSI-1-Ag 4-1

Format: **State:** Lyophilized Ascites (filtered to 0.45 µ (non-sterile))
Buffer Solution: PBS
Preservatives: 0,02% sodium azide
Stabilizers: EIA grade BSA
Reconstitution: Restore with 0.5 ml of cold distilled water.

Applications: Functional Testing (see Protocols).
Cytotoxicity Analysis (see Protocols).
Cytotoxicity Depletion Assay (see Protocols).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

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 (i.e. AKR/J).
Species: Mouse.
Other species not tested.

Storage: Prior to and following reconstitution store the antibody at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References: 1. Krieg, A., Gourley, M. and Steinberg, A. 1991. Association of Murine Lupus and Thymic Full-Length Endeneous Retroviral Expression Maps To A Bone Marrow Stem Cell. J. Immunol. 146:3002-3005.
2. Haba, S. and Nisonoff, A., 1991. Induction of Tolerance To Syngeneic IgE In Neonatal Mice. J. Immunol. 146:807-811.
3. Miyajima, H., Takao, H., et al. 1991. Suppression By IL-2 Of IgE Production By B Cells Stimulated By IL-4. J. Immunol. 146:457- 462.
4. Kruger, M. and Riley, R. 1990. The Age-Dependent Loss Of Bone Marrow B Cell Precursors In Autoimmune NZ Mice Results From
Decreased Mitotic Activity, But Not From Inherent Stromal Cell Defects. J. Immunol. 144:103-110.
5. Fine, J., Siverstone, A. and Gasiewicz, T. 1990. Impairment Of Prothymocyte Activity By 2,3,7,8-Tetrachlorocibenzo-p-Dioxin. J. Immunol. 144:1169-1176.

Protocols: **CYTOTOXICITY ANALYSIS:**

Method:
1. 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 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 Complementc 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 Pictures.

Results - Antibody Titration:
Cell Source: Thymus
Donor: C57BL/6
Cell Concentration: 1.1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10
Procedure: Two-stage cytotoxicity

Results - Tissue Distribution:
Antibody Concentration Used: 1:400
Strain: C57BL/6

Cell Source C.I.
Thymus: 100
Spleen: 14
Lymph Node: 63
Bone Marrow: 4

Results - Strain Distribution
Antibody Concentration Used: 1:80
Strains Tested: C57BL/6, C3H/He, CBA/J, BALB/c, A.TL, AKR/J

Positive: C57BL/6, C3H/He, CBA/J, BALB/c, A.TL
Negative: AKR/J

CYTOTOXICITY DEPLETION ASSAY:

Method:
1. 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®-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 1:500 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 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.

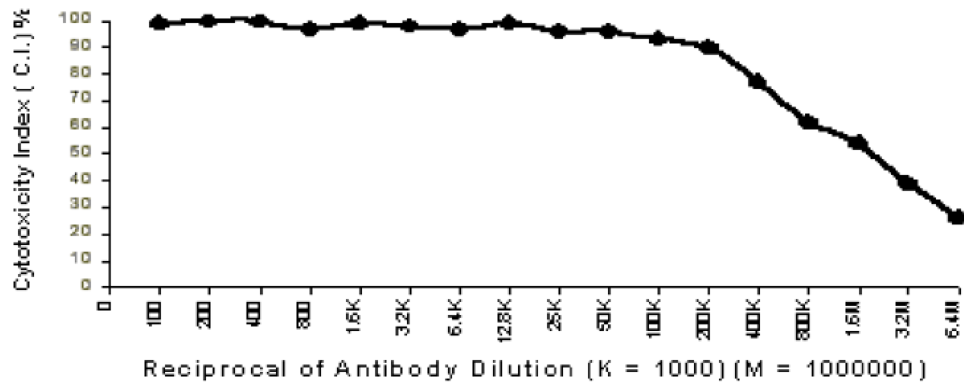
Results:
Cell Source: Splenocytes
Donors: BALB/c and AKR/J
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:500
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10

Treatment of BALB/c splenocytes with this Ab 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 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.

Pictures:



Cytotoxicity Analysis

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

Cytotoxicity Analysis - Cytotoxic Index (C. I.)