CL037
Acris Antibodies GmbH

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

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.: CL037

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 / IgM

Clone: T11D7e

Immunogen: Rat Thymocytes.

Format: **State:** Lyophilized (sterile filtered) ascites.
Reconstitution: Restore with 0.5 ml of cold distilled water.

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

Specificity: This antibody is specific for T cells from mouse strains expressing the Thy 1.1 phenotype (eg. AKR, RF).
It can be used to selectively identify or deplete T cells or their precursors.
Cross reacts with rat Thy 1.

Storage: Store the lyophilized antibody at 2-8°C for one month or at -20°C for longer.
After reconstitution aliquot and store at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols: **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 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 1:80 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 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.

Results-Antibody Titration by Cytotoxicity Analysis:
Cell Source: Thymus
Donor: AKR/J
Cell Concentration: 1.1x10e6 cells/ml Rabbit Complement (Concentration: 1:10).

Tissue Distribution:
Procedure: see before
Antibody Concentration Used: 1/1280
Strain: AKR/J

Cell Source-C.I.
Thymus: 100
Spleen: 16
Lymph Node: 64
Bone Marrow: 9

Strain Distribution:
Procedure: see before
Antibody Concentration Used: 1:20
Strains Tested: AKR/J, C57BL/6, BALB/c, C3H/He
Positive: AKR/J
Negative: C57BL/6, BALB/c, C3H/He

CYTOTOXICITY DEPLETION ASSAY:

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on 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 1:80 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:80 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 Rabbit Complementc diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of 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: AKR/J and BALB/c
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:500
Complement: Rabbit Complement (Concentration Used: 1:10)

Treatment of AKR/J splenocytes with this antibody 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 BALB/c cells were used. These results are consistent with the removal of T helper and T cytotoxic cell activity.

Pictures:

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