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 AP31642SU-N

Thy1 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	CT
Recommended Dilution:	**Cytotoxicity studies** (See Protocols).
Reactivity:	Rat
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Specificity:	This Serum antibody is a Rabbit anti-Rat brain associated Thy1.1 antiserum. This antibody is strongly cytotoxic to Thy1.1 positive lymphocytes. It also cross-reacts with both Mouse Thy 1.1 and Thy 1.2 positive lymphocytes. The Thy 1.1 determinant is expressed primarily on Rat brain and thymus cells but most peripheral Rat T cells lack the Thy 1.1 determinant. Thus, the Thy 1.1 antigen cannot be regarded as a general T cell marker in the Rat.
Formulation:	State: Serum State: Lyophilized Serum
Reconstitution Method:	Restore with 1.0 ml of distilled water.
Conjugation:	Unconjugated
Storage:	Store at -20°C before reconstitution. Aliquot and freeze the unused portion in volumes appropriate for single usage (repeated freezing and thawing may cause loss of antibody activity).
Stability:	Shelf life: one year from despatch.
Gene Name:	Thy-1 cell surface antigen
Database Link:	Entrez Gene 24832 Rat P01830
Synonyms:	Thy-1, THY1, CDw90
Note:	**Sterility:** This reagent is not sold as 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

View online »

medium and filter through a 0.45 µm filter.

Protocol: **Recommended Method For Depleting A Cell Population Of Rat Lymphoctyes:**
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium (1) or equivalent. Remove erythrocytes and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte-Rat (2) density cell separation medium. After washing, adjust the cell concentration to 1.0x10e7 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®-R3 Complement, diluted to the appropriate concentration in Cytotoxicity Medium. Recommended concentration included with each batch of Low-Tox®-R 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 cultured. Layering the cell suspension on separation medium and centrifuging at room temperature as per the instructions provided can do this. 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 then be washed and resuspended in the a ppropriate medium for further processing immediately after step #6, provided that the dead cells will not interfere with subsequent assays.

Recommended Method For Determining Percent Cytotoxicity With Anti-Rat Thy-1.1 Heteroantiserum Plus Complement:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium (1) or equivalent. Remove erythrocytes and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte-Rat (2) density 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 1:40 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®-R Complement (3) diluted to the appropriate concentration in Cytotoxicity Medium (recommended concentration included with each batch of Low-Tox®-R Complement).
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. Cytotoxic Index (C.I.) can be calculated as follows:
C.I. = %Cyt (Ab+Complement) - %Cyt (Complement) / 100% - %Cyt (Complement).

Strain Distribution:
Target Cells: Rat thymus
Procedure: as above
Strains tested: Lewis, Wistar, Fischer, Buffalo
Results: 90-100% of all thymocytes of the strains tested were killed.

Protein Concentration: 78.5 +/- 9.4 mg/ml

Functional Testing:
Cell Source: Splenocytes
Donor: Wistar
Cell Concentration: 1x10e7 cells / ml.
Antibody Concentration: 1:20
Complement: Low-Tox®-R Complement at 1:10

Procedure:
Cells were treated as described in**Recommended Method for depleting a Cell Population of Rat Lymphocytes**. The remaining viable cells were exposed to the mitogens Concanavalin A (Con A), Phytohaemagglutinin (PHA) and Lipopolysaccharide (LPS).
Cell depletion with Anti-Rat Thy-1.1 Serum was found to inhibit the Con A, PHA and LPS responses. (91%, 85%, and 22% respectively).
Treatment of rat splenocytes with Anti-Rat Thy-1.1 Serum plus complement essentially eliminated in vitro T effector cell function.

Cytotoxic Properties:
Target cell source: Wistar thymus, spleen, lymph node, bone marrow.
Target cell concentration: 1.1x10e6 cells/ml
Antiserum concentration: 1:40
Complement: Low-Tox®-R Complement at 1:10

Procedure:
Cytotoxicity studies were carried out using the two stage method as described on page two, with Low-Tox®-R as the source of complement.

Target Cell: C.I.
Thymus: 99
Spleen: 11
Lymph Nodes: 3
Bone Marrow: 50

Antiserum concentration that results in 50% Cytotoxicity against thymus cells: ~ 1:1300.

Notes:
1. Cytotoxicity Medium is RPMI-1640 with 25mM 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.

2. Lympholyte-Rat cell separation medium is a density cell separation medium designed specifically for the isolation of viable rat lymphocytes. This separation medium provides a high and non-selective recovery of viable rat lymphocytes, removing erythrocytes and dead cells. Isolation of rat lymphocytes on cell separation medium of density 1.077 will result in high and selective loss of lymphocytes and should be avoided.

3. Low-Tox®-R complement is a highly active source of complement specifically designed for use with rat lymphocytes. When used in conjunction with Cytotoxicity Medium, this reagent provides a highly potent source of
complement with minimal background toxicity.

Product images: