CL111
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Monoclonal Antibody to CD45 / LCA - Ascites

Alternate names: L-CA, Leukocyte common antigen, PTPRC, Receptor-type tyrosine-protein phosphatase C, T200

Catalog No.: CL111

Quantity: 0.5 ml

Background: CD45 is a family of single chain transmembraneous glycoproteins consisting of at least four isoforms (220, 205, 190, 180 kDa) which share a common large intracellular domain. Their extracellular domains are heavily glycosylated. The different isoforms are produced by alternative messenger RNA splicing of three exons of a single gene on chromosome 1. CD45 is expressed on cells of the human hematopoietic lineage (including hematopoietic stem cells) with the exception of mature red cells. It is not detected on differentiated cells of other tissues. It is likely that CD45 plays an important role in signal transduction, inhibition or upregulation of various immunological functions. Antibodies recognising a common epitope on all of the isoforms are termed CD45 whilst those recognising only individual isoforms are termed CD45RA or CD45RO etc.

Uniprot ID: P04157

NCBI: NP_001103357.1

GeneID: 24699

Host / Isotype: Mouse / IgG2a

Clone: OX-30

Immunogen: Immunogen:Lymph Node glycoproteins and cells.
Donor: BALB/c Spleen.
Fusion Partner: NSO/U.

Format: **State:** Lyophilised Ascites
Reconstitution: Reconstitute with 0.5 ml of cold distilled water.

Applications: Flow Cytometry.
Immunohistochemistry on frozen sections.
(Reported to be unsuitable for use with paraffin sections.)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

This monoclonal antibody recognizes a monomorphic determinant of the rat leukocyte common antigen.(1) The antigen recognized is a heavily glycosylated membrane glycoprotein of molecular weight 170 kDa on thymocytes but molecular weight 170-220 kDa on other leukocytes. The leukocyte common antigen (L-CA) is a major glycoprotein of haematopoietic cells but is not found on other tissues or erythroid cells. It is present on greater than 95% of thymocytes, bone marrow cells and thoracic duct lymphocytes. This molecule carries much of the carbohydrate of thymocytes and shows interesting heterogeneity amongst T lymphocytes and B lymphocytes.
Species: Rat.
Other species not tested.

Storage:

Prior to reconstitution store at 2-8°C.
Following reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References:

1. Sunderland,C.A., McMaster, W.R., and A.F. Williams. (1979) Eur. J.Immunol. 9, 155-159. Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes.
2. Brown,W.R.A., Barclay,A.N., Sunderland,C.A. and A.F. Williams. (1981) Nature. 289, 1164-1177. Identification of a glycoprotein-like molecule at the cell surface of rat thymocytes.
3. Standring,R. and A.F. Williams. (1978) Biochim.Biophys. Acta. 508, 85-96. Glycoproteins and antigens of membranes prepared from rat thymocytes after lysis by shearing or with the detergent Tween-40.
4. Brown,W.R.A. and A.F. Williams. (1982) Immunology. 46, 713-726. Lymphocyte cell surface glycoproteins which bind to soybean and peanut lectins.
5. Woollet,G.R., Barclay,A.N., Puklavec,M. and A.F. Williams. (1985) Eur. J. Immunol. 15, 168-173. Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes.
6. Brouard, S., et al. (1999) J. of Immunol. 162, 3367-3377. T Cell repertoire alterations of vascularized xenografts.

Protocols:

FLOW CYTOMETRY ANALYSIS:

METHOD:

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat cell separation medium.
2. Wash 2 times.
3. Resuspend cell to 1x10e6 cells in approximately 50 µl Media A in a microcentrifuge tube. (i.e.. 50 µl of cells resuspended to 2x10e7 cells/ml).
4. To each tube add 50 µl of a 1:1 000-1:10 000 dilution of this Ab.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-mouse IgG (H+L)) at 1:500 dilution.
9. Incubate tubes at 4°C for 30-60 minutes.
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 phosphate buffered saline. (This stains dead cells by intercalating DNA).

MEDIA:

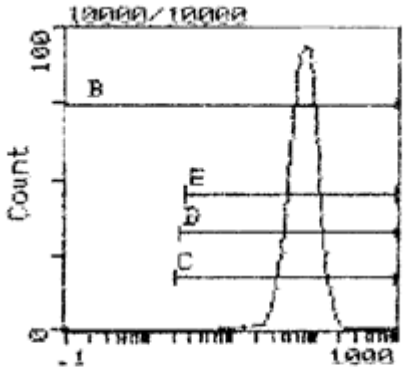
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2 M sodium azide in 100 mls.)
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls.)

RESULTS:
Rat Strain: Lewis Rat
Cell Concentration: 1x10e6 cells per test
Antibody Concentration: 1:2000
Isotypic Control: Mouse IgG2a

CELL SOURCE PERCENT STAINING
Thymus: *99.9%*
Spleen: *99.9%*
Lymph Node: *99.9%*

RESULTS - STRAIN DISTRIBUTION:
Antibody Concentration: 1:2000
Strains Tested: Lewis, Wistar, ACI, Brown Norway, Buffalo, Fischer 344
Positive: Wistar, Buffalo, Brown Norway, ACI, Fischer 344, Lewis
Negative: none

Pictures:



LFL1
Cell Source: Thymus
Percentage of Cells Stained Above Control: 99.9%