

Monoclonal Antibody to CD45 / LCA - Purified

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

T200

Catalog No.: CL111PX
Quantity: 0.5 mg
Concentration: 1.0 mg/ml

Background: The antigen recognized is a heavily glycosylated membrane glycoprotein of molecular

weight 170,000 kDa on thymocytes but molecular weight 170,000-220,000 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

Uniprot ID: P04157

NCBI: NP 001103357.1

GenelD: 24699

Host / Isotype: Mouse / IgG2a

Clone: OX-30

Immunogen: Lymph node glycoproteins and cells.

Donor: BALB/c spleen Fusion Partner: NSO/U

Format: State: Liquid purified Ig

Purification: Protein G Chromatography **Buffer System:** PBS and 0.02% NaN3

Applications: Flow cytometry (see protocol).

Immunohistochemistry with frozen 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. **Species:** Rat.

Other species not tested.

Storage: 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.

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- 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
 - 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 a 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 the cells to a concentration of 2x10e7 cells/ml in media A. Add 50µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
- 4. To each tube, add 0.5-1.0 μg* 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 1:700 dilution.
- 9. Incubate the tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
- 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 PBS. This stains dead cells by intercalating in DNA.

Media:

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

Results - Tissue Distribution:

Rat Strain: Wistar

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.5 µg/10e6 cells

<u>Isotypic Control</u>: Mouse IgG2a

Cell Source Percentage of cells stained above control:

Thymus: 99.9% Spleen: 97.4% Lymph Node: 90.6%

For research and in vitro use only. Not for diagnostic or therapeutic work.

Material Safety Datasheets are available at www.acris-antibodies.com or on request.



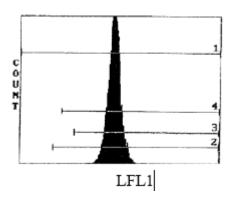
Results - Strain Distribution:

Antibody Concentration Used: 0.5 µg/10e6 cells

Strains Tested: Wistar, Buffalo, Brown Norway, Fischer 344
Positive: Wistar, Buffalo, Brown Norway, Fischer 344

Negative: none

Pictures:



Cell Source: Thymus - Percentage of cells stained above control: 99.9%