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Monoclonal Antibody to CD45 / LCA - Purified

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

Catalog No.: CL111P

Quantity: 0.25 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

GeneID: 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.

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 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.

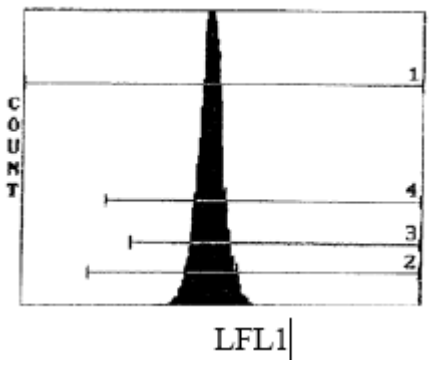
Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results - Tissue Distribution:
Rat Strain: Wistar
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.5 µg/10e6 cells
Isotypic Control: Mouse IgG2a

Cell Source Percentage of cells stained above control:
Thymus: 99.9%
Spleen: 97.4%
Lymph Node: 90.6%

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%