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Monoclonal Antibody to CD45 / LCA - PE

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

Catalog No.: CL111R

Quantity: 50 µg

Concentration: 0.1 mg/ml

Background: 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. (2,3).

Uniprot ID: P04157

NCBI: NP_001103357.1

GeneID: 24699

Host / Isotype: Mouse / IgG2a

Clone: OX-30

Immunogen: Lymph node glycoproteins and cells.

Format:
State: Liquid purified IgG fraction.
Purification: Protein G Chromatography.
Buffer System: PBS with 0.02% Sodium Azide as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.
Label: PE – Conjugated to

Applications:
Suitable for use in Flow cytometry (See Protocol).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This antibody recognizes a monomorphic determinant of the rat leukocyte common antigen (CD45) (1). 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.
Species: Rat.
Other species not tested.

Storage:
Store the antibody at 2-8°C.
DO NOT FREEZE!
Avoid prolonged exposure to light.
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 Rat cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube, 0.5 µg-1.0 µg of CL111R or CL111RX per 10e6 cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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: (Figure 1)
Rat Strain: Wistar
Cell Concentration: 1x10e6 cells per test.
Antibody Concentration Used: 0.5 µg/10e6 cells.
Isotypic Control: PE Mouse IgG2a.

Cell-Source Percentage of cells stained above control:
Thymus: 99.8%
Spleen: 97.1%
Lymph Node: 98.9%

Strain Distribution:

Strains Tested: Wistar, Buffalo, Brown Norway, Fischer 344
Positive: Wistar, Buffalo, Brown Norway, Fischer 344
Negative: none

Pictures:

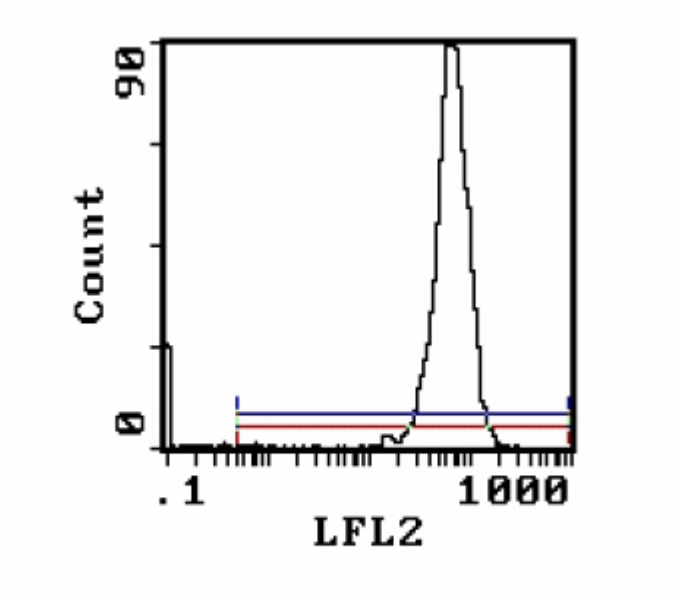

Figure 1. Results-Tissue Distribution.