Acris Antibodies GmbH

Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

US office:
Acris Antibodies, Inc.
San Diego, CA
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

Monoclonal Antibody to CD45 / LCA (CD45R) - APC

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

Catalog No.: SM034APC

Quantity: 0.1 mg

Concentration: 0.2 mg/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: P06800

NCBI: 10090

Host / Isotype: Rat / IgG2a

Clone: RA3-6B2

Immunogen: Mouse pre-B tumour cells

Format:
State: Liquid purified Ig fraction
Buffer System: PBS, 0.09% sodium azide (NaN3) and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.
Label: APC

Applications:
Flow cytometry (for details please see specificity / protocols).
Flow cytometry.
Immunoprecipitation.
Immunohistochemistry on frozen and paraffin sections.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

This antibody reacts with an exon A-specific epitope on CD45 that is expressed on essentially all B cells and is maintained throughout B cell development (1,6). The RA3-6B2 epitope is expressed in a restricted manner by B cells; however, it is also present on lymphokine activated killer (LAK) cells (1). Although B220 has been widely used as a pan B cell marker, CD19 may be a more appropriate pan B cell marker, as expression of CD19 appears to be more closely restricted to B cells. It has been shown to potentiate isotype switching in B cells and to inhibit proliferative responses of B cell mitogens on B cells (7,8).
This antibody immunoprecipitates the high molecular weight (220,000 Da) surface molecule of the leukocyte common antigen B220 on B cells (1).
Tissue Distribution by Flow Cytometry Analysis: (Representative Histogram)
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.5 µg/10e6 cells
Isotypic Control: APC Rat IgG2a.
Species: Mouse.
Other species not tested.

Storage:

Store the antibody at 2 - 8 °C. DO NOT FREEZE! This product is photosensitive and should be protected from light.
Shelf life: one year from despatch.

General References:

1) Coffman, B. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. Immunological Rev. 69:5 - 23.
2) Zuhair, K., Ballas, and Rasmussen, W., 1993. Lymphokine-activated killer cells VII. IL-4 induces an NK1.1 + CD8a+b- TCR αβ B220+ lymphokine-activated killer subset.
3) Asensi, V., and Kimeno, K., et al. 1989. Treatment of autoimmune MRL/1pr mice with anti-B220 monoclonal antibody reduces the level of anti-DNA antibodies and lymphadenopathies. Immunology 68:204 - 208.
4) Ballas, A. K., and W. Rasmussen. 1990. Lymphokine-activated killer (LAK) cells. IV. Characterization of murine LAK effector subpopulations, J. Immunol. 144:386.
5) Whiteland, J.L et al (1995). Immunohistochemical detection of T cell subsets and other leukocytes in paraffin embedded rat and mouse tissues with monoclonal antibodies .J. Histochem. Cytochem. 43: 313-320.
6) Johnson, P., A. Maiti, and D.H.W. Ng. 1997. CD45: A family of leukocytespecific cells surface glycoproteins. In Wier's Handbook of Experimental Immunology. Vol. 2. L.A. Herzenberg, D.M. Weir, and C. Blackwell, eds. Blackwell Science, Cambridge, MA, pp.62.1-62.16.
7) George, A., S. Rath, K.E. Shroff, M. Wang, and J.M. Durdik. 1994. Ligation of CD45 on B cells can facilitate production of secondary Ig isotypes. J. Immunol. 152: 1014-1021.
8) Domiati-Saad, R., E.W. Ogle, and L.B. Justement. 1993. Administration of anti-CD45 mAb specific for B cell-restricted epitope abrogates the B cell response to a T-dependent antigen in vivo. J. Immunol. 151: 5936-5947.

Protocols:

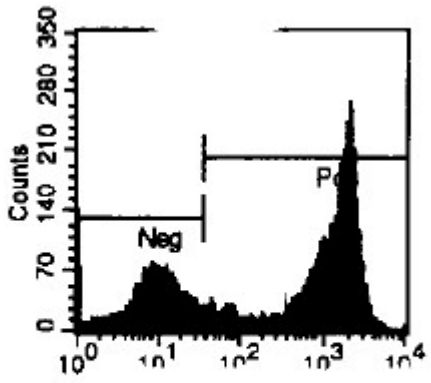
FLOW CYTOMETRY ANALYSIS:
Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population.
2. Wash 2 times.
3. Re-suspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube, add ~0.5 µg of antibody 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. Re-suspend 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).

Pictures:



LFL4

Percentage of cells stained above control (R2) = 36.96%
Cell Source: Spleen