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Monoclonal Antibody to CD44 - Purified

Alternate names: CDw44, ECMR-III, Epican, Extracellular matrix receptor III, GP90 lymphocyte homing/adhesion receptor, HUTCH-I, Heparan sulfate proteoglycan, Hermes antigen, Hyaluronate receptor, LHR, MDU2, MDU3, MIC4, PGP-1, Phagocytic glycoprotein 1

Catalog No.: CL110PX

Quantity: 0.5 mg

Concentration: 1.0 mg/ml

Background: This antigen is expressed on most leukocytes (except a sub population of B cells) and increases upon activation. This antibody binds extracellularly to the standard (S) form on rat leukocytes, but it is not known if they bind to the N-terminal region. It has also been reported that the antibody may bind to melanoma cell lines that express CD44V (splice variant form).
CD44 is expressed on most leukocytes except a sub population of B cells. Its expression is increased on T and B blasts.

Uniprot ID: P26051

NCBI: 10116

Host / Isotype: Mouse / IgG2a

Clone: OX-49

Immunogen: T cell blasts.

Format: **State:** Liquid purified IgG fraction.
Purification: Protein G affinity chromatography.
Buffer System: PBS buffer with 0.02% sodium azide as preservative.

Applications: This antibody is suitable for Immunoprecipitation, Flow cytometry (See protocol), Western Blotting and Immunohistochemisty on frozen and paraffin sections.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Reacts with rat CD44 (Pgp-1).
This antibody recognizes an epitope on both standard CD44 and its splice variant.
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. Patterson, D.J., et al. 1987 Antigens of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. Molec. Immunol. 24(12): 1281-1290.
2. Arch, R., et al. 1992. Participation in normal immune response of a metastases inducing

splice variant of CD44. Science. 257:682-685.
3. Wang, H., et al. 2001. Use of suppression subtractive hybridization for differential gene expression in stroke: discovery of CD44 gene expression and localization in permanent focal stroke in rats. Stroke. 32: 1020-1027.
4. Jain, M., et al. 1996. Role of CD44 in the reaction of vascular smooth muscle cells to arterial wall injury. J. Clin. Invest. 97(3): 596-603.
5. Lewington, A.J.P., et al. 2000. Expression of CD44 in kidney after acute ischemic injury in rats. Am. J. Physiol. 278: R247-R254.
6. Foster, L.C., et al. 1998. Regulation of CD44 gene expressionby the proinflammatory cytokine interleukin-1b in vascular smooth muscle cells. J. Biol. Chem. 273(32): 20341-20346.

Protocols:

FLOW CYTOMETRY ANALYSIS:

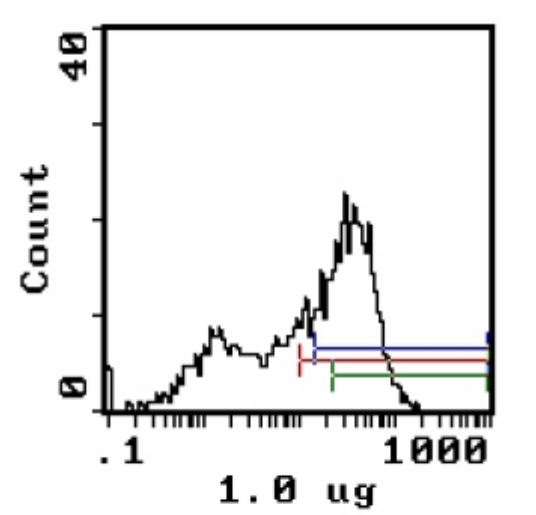
Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Rat cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 1.0-0.5 µg* of CL110P or CL110PX.
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 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: 1 x 10e6 cells per tests
Antibody Concentration Used: 0.2 µg/10e6 cells
Isotypic Control: Mouse IgG2a.
Cell Source-Percentage of cells stained above control:
Thymus: 82.1%
Spleen: 53.5%
Lymph Node: 87.1%

N.B. Appropriate control samples should always be included in any labelling studies.
* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.

Pictures:



Cell Source: Spleen
Percentage of cells stained above control: 53.5%