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Monoclonal Antibody to CD44 - FITC

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.: CL110FX

Quantity: 0.5 mg

Concentration: 0.1 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 with 0.02% sodium azide as preservative and EIA grade BSA as a stabilizer
Label: FITC

Applications:
Flow cytometry (See protocol).
Immunohistochemistry on Frozen Sections
Immunohistochemistry on Paraffin Sections.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This anti-Rat CD44 monoclonal antibody recognizes an epitope on both standard CD44 and its splice variant.

Species Reactivity: **Tested:** Rat

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.
7. 4. Stroke. 32: 1020-1027.

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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 1.0-0.5 µg* of CL110F or CL110FX.
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).

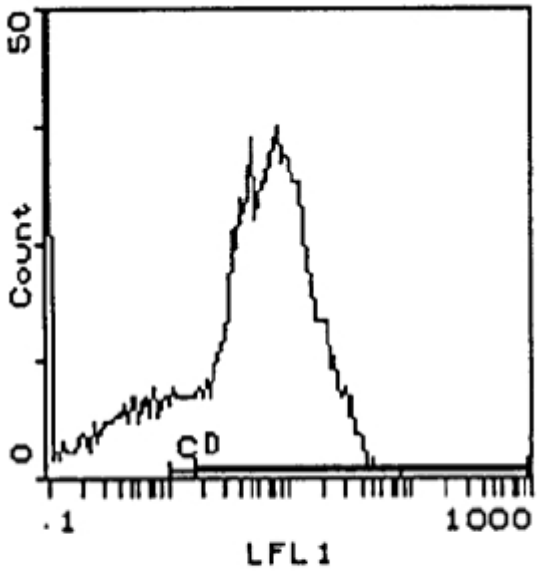
Results-Tissue Distribution:
Rat Strain: Wistar
Cell Concentration: 1 x 10e6 cells per tests
Antibody Concentration Used: 1.0 µg/10e6 cells
Isotypic Control: FITC Mouse IgG2a.

Cell Source Percentage of cells stained above control:
Thymus: 99.6%
Spleen: 75.9%
Lymph Node: 96.8%

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: 75.9%