

## Monoclonal Antibody to CD44 - Purified

<b>Alternate names:</b>	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
<b>Catalog No.:</b>	CL023P
<b>Quantity:</b>	0.25 mg
<b>Concentration:</b>	1,0 mg/ml
<b>Background:</b>	CD44 is a type 1 transmembrane glycoprotein also known as Phagocytic Glycoprotein 1 (pgp 1) and HCAM. CD44 is the receptor for hyaluronate and exists as a large number of different isoforms due to alternative RNA splicing. The major isoform expressed on lymphocytes, myeloid cells, and erythrocytes is a glycosylated type 1 transmembrane protein. Other isoforms contain glycosaminoglycans and are expressed on hematopoietic and non hematopoietic cells. CD44 is involved in adhesion of leukocytes to endothelial cells, stromal cells, and the extracellular matrix.
<b>Uniprot ID:</b>	<a href="#">P15379</a>
<b>NCBI:</b>	<a href="#">NP_001034240.1</a>
<b>GeneID:</b>	<a href="#">12505</a>
<b>Host / Isotype:</b>	Rat / IgG2a
<b>Clone:</b>	KM81
<b>Immunogen:</b>	Bone Marrow Derived Stromal Cells (clone BMS2). Donor: Lou/MN Rat. Fusion Partner: SP 2/0.
<b>Format:</b>	<b>State:</b> Liquid purified Ig fraction. <b>Purification:</b> Protein G Chromatography. <b>Buffer System:</b> PBS containing 0.02% Sodium Azide as preservative.
<b>Applications:</b>	Flow Cytometry Analysis (see Protocol). Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

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**Specificity:** This monoclonal antibody recognizes a 95 kDa glycoprotein found on most hematopoietic cells (1). It is thought to be important in the regulation of migratory properties of lymphocytes during development and the regulation of the interaction with bone marrow stromal cells during hematopoiesis (2,3). CD44 functions as a receptor for hyaluronate, although some cells expressing CD44 do not bind hyaluronate (3,4). This antibody has been shown to inhibit the growth of lymphoid and myeloid cells in long term bone marrow cultures (3). It also blocks the adhesive interactions of B cell hybridomas to a cloned stromal line or to hyaluronate coated dishes (4).

**Species:** Mouse.  
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. Lynch, F., and Ceredig R. Ly-24 (Pgp-1) expression by thymocytes and peripheral T cells. Immunol. Today 9:7.0.  
2. Picker, L.J., De Pos Toyos J, Telen MJ et al. Monoclonal antibodies against CD44 [In (Lu)-related P80], and Pgp-1 antigens in man recognize the Hermes class of lymphocyte homing receptors. J. Immunol. 1989; 142:2046-51.  
3. Miyake K, Medina K, Hayashi S-I et al. Monoclonal antibodies to Pgp-1/CD44 block lympho-hemopoiesis in long term bone marrow cultures. J. Exp. Med. 1990; 171:477-488.  
4. Miyake K, Underhill CB, Lesley J. et al. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. J. Exp. Med 1990; 172:69-75.

**Protocols:** **FLOW CYTOMETRY ANALYSIS:**

**Method:**

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M 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 0.5 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-rat 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:**

Mouse Strain: BALB/c

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.5 µg/10e6 cells

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Isotypic Control: Rat IgG2a

**Results - Strain Distribution:**

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.5 µg/10e6 cells

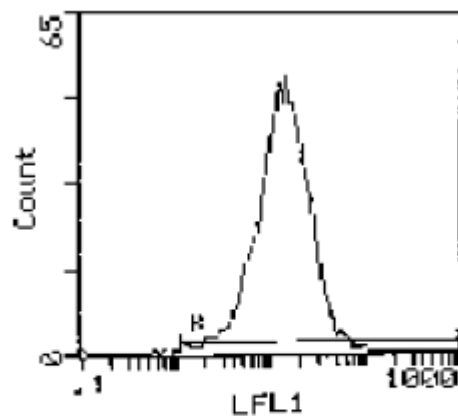
Strains Tested: BALB/C, CBA/J, C3H/He, C57BL/6, SWR

Positive: BALB/C, CBA/J, C3H/He, C57BL/6, SWR

Negative: none

**Pictures:**

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	79.6%
Spleen	99.1%
Lymph Node	81.2%
Bone Marrow	80.2%



Cell Source: Spleen  
Percentage of cells stained above control: 99.1%

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