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Product datasheet for CL025

Cd44 Mouse Monoclonal Antibody [Clone ID: 5034-44.2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5034-44.2
Applications:	CT, FC
Recommended Dilution:	Flow cytometry.(see Protocols) Method for Determining percent of positive cells in a poulation. (see Protocols) Method for Depleting a cells poulation of positive lymphocytes. (see Protocols)
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	B6 - Ly-1a spleen DONOR: BALB/c spleen FUSION PARTNER: P3-NS1-1-Ag4(NS1/1)
Specificity:	This monoclonal antibody recognizes the murine alloantigen Ly 24.2. CD44 (Ly 24B.2, Pgp-1.2) is a 95 kDa glycoprotein previously known as phagocytic glycoprotein -1. It has a wide tissue distribution and is found on bone marrow derived cells, lymphocytes and non - lymphoid tissue such as brain, liver, and kidney. There is variation in Ly 24 expression between mice strains. Generally expression by Ly 24.1 strains is high while in Ly 24.2 it is lower (2). The Ly 24 antigen is expressed by T lymphocytes during primary antigen stimulation. This antigen can be used as a marker to identify activated or memory T cells (3,4).
Formulation:	State: Ascites State: Lyophilised Ascites
Reconstitution Method:	Reconstitute with 0.5 ml of cold distilled water.
Conjugation:	Unconjugated
Storage:	Prior to and following reconstitution store the antibody at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD44 antigen

View online »

Database Link: Entrez Gene 12505 Mouse
P15379

Background: 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.

Synonyms: LHR, MDU2, MDU3, MIC4, CDw44, Epican, ECMR-III, HUTCH-I, Heparan sulfate proteoglycan, Hermes antigen, Hyaluronate receptor, PGP-1

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:
1. Prepare 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 cells to 1 x 10e6 cells in approximately 50 µl Media A in a microcentrifuge tube (ie. 50 µl of cells resuspended to 2 x 10e7 cells / ml). (THE CONTENTS OF 1 TUBE REPRESENTS 1 TEST).
4. To each tube add 50 µl of 1/500 dilution of this Ab(final dilution 1/1000).
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 CLCC30201 (Goat anti-mouse IgG(H+L)-FITC conjugate) @ 1:700.
9. Incubate 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 phosphate buffered saline. (This stains dead cells by intercalating 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:
PROCEDURE: as above

ANTIBODY CONCENTRATION: 1:1000
MOUSE STRAIN: C57BL/6

CELL SOURCE: PERCENT STAINING
Thymus: 8.8%
Spleen: 36.1%
Lymph Node: 17.9%

RESULTS - STRAIN DISTRIBUTION:
PROCEDURE: as above
ANTIBODY CONCENTRATION: 1:1000
STRAINS TESTED: C57BL/6, BALB/c, AKR, C3H/He, CBA/J
POSITIVE: C57BL/6, AKR, C3H/He, CBA/J
NEGATIVE: BALB/c

RECOMMEND METHOD FOR DEPLETING A CELL POPULATION OF CD44 (Ly 24B.2, Pgp-1.2) POSITIVE LYMPHOCYTES:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1x10e7 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:500 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:500 in Cytotoxicity Medium.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox®-M Rabbit Complement, diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox®-M Rabbit
Complement).
6. Incubate for 60 minutes at 37°C.
7. Monitor for percent cytotoxicity at this stage, before further processing. For this purpose remove a small sample from each tube, dilute 1:10 with medium, and add 1/10 volume of 1% trypan blue. After 3-5 minutes, score live vs. dead cells in a hemacytometer.
8. For functional studies, remove the dead cells from the treated groups before further processing, particularly if the treated cells are to be cultured. This can be done by layering the cell suspension separation medium and centrifuging at room temperature as per the instructions provided. Live cells will form a layer at the interface, while the dead cells pellet. The interface can then be collected and washed in Cytotoxicity Medium before being resuspended in the appropriate medium for further processing.
Alternately, the cells can be washed and resuspended in the appropriate medium for further processing immediately after Step #6, provided that the dead cells will not interfere with subsequent assays.

RECOMMENDED METHOD FOR DETERMINING PERCENT OF CD44 (Ly 24B.2, Pgp-1.2)

POSITIVE CELLS IN A POPULATION.

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1 x 10e6 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:1000 and mix.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox®-M Rabbit Complement3 diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox®-M Rabbit Complement).
6. Incubate for 60 minutes at 37°C.
7. Place on ice.
8. Add trypan blue. 10% by volume of 1% trypan blue (w/v) added 3-5 minutes before scoring works well. Score live vs dead cells in a hemacytometer.
9. Cytotoxic index see pictures

NOTES:

1. Cytotoxicity Medium is RPMI-1640 with 25mM Hepes buffer and 0.3% bovine serum albumin (BSA). BSA is substituted for the conventionally used fetal calf serum (FCS) because we have found that many batches of FCS contain complement dependent cytotoxins to mouse lymphocytes, thus increasing the background killing in the presence of complement. We recommend that cells not be exposed to FCS prior to or during exposure to antibody and complement. Some batches of BSA also contain complement dependent cytotoxins, resulting in the same problem.
We screen for batches of BSA giving low background in the presence of complement and use the selected BSA for preparing Cytotoxicity Medium.
2. Lympholyte®-M cell separation medium is density separation medium designed specifically for the isolation of viable mouse lymphocytes. This separation medium provides a high and non-selective recovery of viable mouse lymphocytes, removing red cells and dead cells. The density of this medium is 1.087 - 1.088. Isolation
of mouse lymphocytes on cell separation medium density 1.077 will result in high and selective loss of lymphocytes and should be avoided.
3. Rabbit serum provides the most potent source of complement for use with antibodies to mouse cell surface antigens. However, rabbit serum itself is very toxic to mouse lymphocytes. Low-Tox®-M Rabbit Complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

RESULTS - TISSUE DISTRIBUTION:

Strain: C57BL / 6
Cell Concentration: 1 x 10e6 cells per ml.
Complement: Low-Tox®-M Rabbit Complement

Complement Concentration: 1:15
Antibody Concentration: 1:1000

CELL SOURCE: C.I.
Spleen: 8
Splenic T-cells: 36
Lymph Node: 14
Lymph Node T-cells: 21
Thymus: 9

PROCEDURE:
Cells were treated as described in Recommended Method for Depleting a Cell Population of Ly 24B.2 (Pgp-1.2) Positive Lymphocytes. Treated cells and controls were tested for (a) the ability to generate plaque-forming cells (PFC) using a modified Jerne haemolytic plaque assay and (b) the ability to generate cytotoxic T effector cells using a cytotoxic lymphocyte reaction (CTL) assay. Cells were treated both before and after sensitization in the CTL assay. In vitro immunizations were used in all experiments.

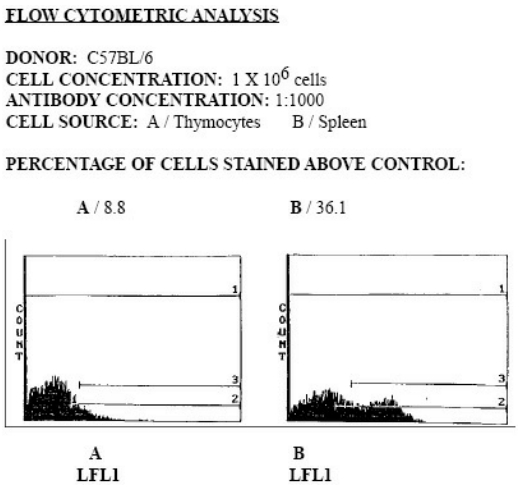
RESULTS:
Treatment of C57BL/6 splenocytes with anti-CD44 (Ly 24B.2, Pgp-1.2) monoclonal antibody plus complement resulted in a marked reduction in the number of plaque-forming cells. Cytotoxic T cell function as assessed by the CTL assay was reduced by approximately 50% in both presensitized and postsensitized treated samples. Treatment of Balb/c splenocytes had little or no effect on the number of plaque-forming cells as well as the cytotoxic T cell - function.

MITOGEN RESPONSE:
Cell Source: C57BL/6 splenocytes
Cell Concentration: 1.1x10e7 cells / ml.
Antibody Concentration: 1:20
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10

PROCEDURE:
C57BL/6 splenocytes were treated as described on page 4 Recommended Method for Depleting a Cell Population of CD44 (Ly 24B.2, Pgp-1.2) Positive Lymphocytes. Remaining viable lymphocytes were exposed to the mitogens Concanavalin A (CON A), Phytohaemagglutinin (PHA) and Lipopolysaccharide (LPS).

RESULTS:
Cell depletion with Anti-CD44 (Ly 24B.2, Pgp 1.2) Monoclonal Antibody had little effect on the LPS response and largely eliminated the CON A and PHA response (84% and 71%, respectively).

Product images:



$$C.I. = \frac{\%\ cyt\ (antibody + complement) - \%\ cyt\ (complement\ alone)}{100\ \% - \%\ cyt\ (complement\ alone)} \times 100$$

Cytotoxic index

Figure : Ly-24 alleles of mice strains

Strain	Ly-24 allele
C57BL/6	2
CBA/H	2
AKR	2
SJL	2
B10.D2	2
BALB/c	1
DBA/2J	1