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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Mouse CD44
Monoclonal Antibody - Ascites

CL8924A
LOT: 0429

DESCRIPTION:

Cedarlane's anti-mouse CD44 (Ly 24B.2, Pgp-1.2) 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).

PRESENTATION:

0.5 ml, lyophilized

STORAGE AND RECONSTITUTION:

Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of cold distilled water. Aliquot and freeze the unused portion in volumes appropriate for single use (to avoid repeated freezing and thawing). If slight turbidity occurs, clarify by centrifugation before use.

For more information or to place an order please contact...

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SPECIFICATIONS:

CLONE: 5034-44.2

HYBRIDOMA PRODUCTION:

IMMUNIZATION:

IMMUNOGEN: B6 - Ly-1^a spleen
IMMUNOCYTE DONOR: BALB/c spleen
FUSION PARTNER: P3-NS1-1-Ag4(NS1/1)

SPECIFICITY: Mouse CD44

Ig CLASS: Mouse IgG_{2a}

PRESENTATION: Ascitic fluid (lyophilized)

FLOW CYTOMETRY ANALYSIS:

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte® M Cell Separation Medium (CL5030).
2. Wash 2 times.
3. Resuspend cells to 1 x 10^6 cells in approximately 50 µl Media A in a microcentrifuge tube (ie. 50 µl of cells resuspended to 2 x 10^7 cells / ml).
   (THE CONTENTS OF 1 TUBE REPRESENTS 1 TEST).
4. To each tube add 50 µl of 1/500 dilution of **CL8924A** (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).

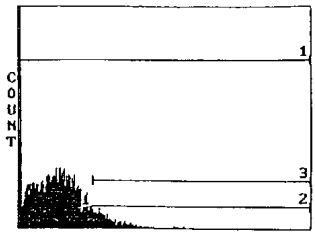
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

FLOW CYTOMETRIC ANALYSIS

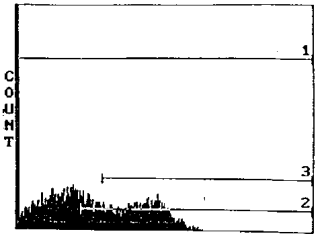
DONOR: C57BL/6
CELL CONCENTRATION: 1 X 10^6 cells
ANTIBODY CONCENTRATION: 1:1000
CELL SOURCE: A / Thymocytes B / Spleen

PERCENTAGE OF CELLS STAINED ABOVE CONTROL:

A / 8.8 **B** / 36.1



A
LFL1



B
LFL1

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.**

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

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 Cedarlane Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M density cell separation medium (CL5030). After washing, adjust the cell concentration to $1x10^7$ 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 Cedarlane Low-Tox®-M Rabbit Complement (CL3051), 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 Cedarlane Cytotoxicity Medium or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M density cell separation medium (CL5030). After washing, adjust the cell concentration to 1 x 10^6 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 Cedarlane Low-Tox®-M Rabbit Complement[3] (CL3051) 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. Cytotoxic index (C.I.) can be calculated as follows:

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

Figure 1: 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

from: Lesley, J. and Trowbridge, I.S. 1982. Immunogenetics 15:313.

NOTES:

1. Cedarlane Cytotoxicity Medium (CL95100) 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 Cedarlane Cytotoxicity Medium (CL5030).
2. Cedarlane 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. Cedarlane Low-Tox®-M Rabbit Complement (CL3051) is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cedarlane Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

TISSUE DISTRIBUTION:

Strain: C57BL / 6
Cell Concentration: 1 x 10^6 cells per ml.
Complement: Cedarlane Low-Tox®-M Rabbit Complement (CL3051)
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

FUNCTIONAL TESTING:

Cell Source: Splenocytes
Donor Strains: BALB/c, C57BL/6.
Cell Concentration: 1 x 10^7 cells / ml
Antibody Concentration: 1:20
Complement: Cedarlane Low-Tox®-M Rabbit Complement (CL3051)
Complement Concentration: 1:10

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: $1.1x10^7$ cells / ml.
Antibody Concentration: 1:20
Complement: Cedarlane Low-Tox®-M Rabbit Complement (CL3051)
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).

REFERENCES

1. Sutton, V.R, G.L. Wijffels, I.D. Walker, P.M. Hogarth, and I.F.C. McKenzie. 1987. Genetic and Biochemical Characterization of Antigens Encoded by the Ly-24 (Pgp-1) locus. J. Immunogenetics 14:43.
2. Lynch F. and R. Ceredig. 1989. Mouse strain variation in Ly-24 (Pgp-1) expression by peripheral T Cells and Thymocytes: Implications for T Cell differentiation. Eur. J. Immunol. 19:223.
3. Budd, R.C, J.C. Cerottini, C. Hovarth, C. Bron, T. Pedrazzini, R.C. Howe. 1987. Distinction of virgin memory T Cells. Stable acquistion of the Pgp-1 glycoprotein concomittant with antigenic stimulation. J. Immunol. 138: 3120.
4. Butterfield, K., C.G, Fathman, R.C. Budd. 1989. A subset of memory $CD4^+$ helper T lymphocytes identified by expression of Pgp-1. J. Exp. Med. 169:1461.

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