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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Rat CD44
Monoclonal Antibody-Ascites

CL044A
LOT: 4411

DESCRIPTION:

Cedarlane's anti-rat CD44 (OX-49) monoclonal antibody recognizes rat CD44 (Pgp-1), also called CD44H. This antigen is expressed on most leukocytes (except a sub population of B cells) and increases upon activation. The OX-49 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).

This antibody is suitable for immunoprecipitation, flow cytometry, Western Blotting (~90 kDa) and immunohistochemisty on frozen and paraffin embedded sections.

PRESENTATION: 0.5 ml, lyophilized ascites

STORAGE/STABILITY:

Lyophilized form stable short term at 4°C, or long term at -20°C. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

SPECIFICATIONS:

Clone: MRC OX-49

Hybridoma Production:

Immunization: Immunogen: T cell blasts
Donor: BALB/c spleen

Fusion Partner: myeloma cell line NSO/1

Specificity: Rat CD44

Ig Class: Mouse IgG_{2a}

Format: Ascitic fluid filtered to 0.45 µ (non-sterile) and lyophilized

Continued overleaf…

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

CEDARLANE® LABORATORIES LIMITED

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FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat cell separation medium (CL5040).
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 50 µl of a 1:500-1:1000 dilution ***** of **CL044A**.
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** (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:

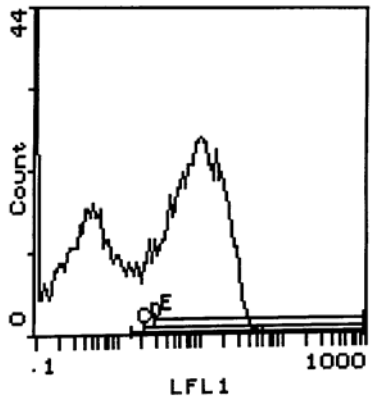
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Fischer
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 1:1000 in 50 µl /10^6 cells
Isotypic Control: Mouse IgG_{2a}

Cell Source	Percentage of cells stained above control:
Thymus	87.8%
Spleen	53.3%
Lymph Node	80.1%
Bone Marrow	54.6%



Cell Source: Spleen
Percentage of cells stained above control: 53.3 %

N.B. Appropriate control samples should always be included in any labelling studies.

Continued overleaf…

REFERENCES:

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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.
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