SZABO SCANDIC

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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# TECHNICALLY Speaking

**Place your order with CEDARLANE® or your local distributor.**

*Please contact CEDARLANE® for lot specific information.*

## Biotin Anti-Rat CD44 Monoclonal Antibody

**CL044B**
**CL044B-5**
**LOT: 4441**

**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 flow cytometry, Western Blotting and immunohistochemisty on frozen and paraffin sections.

**PRESENTATION:**

100 µg (CL044B) or 500 µg (CL044B-5) Biotin conjugated Ig buffered in PBS, 0.02% $NaN_3$ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

**STORAGE/STABILITY:**

Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.

**SPECIFICATIONS:**

Clone: MRC OX-49

Hybidoma Production:

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

Fusion Partner: myeloma cell line NSO/1

Specificity: Rat CD44

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

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**website: www.cedarlanelabs.com** • e-mail: info@cedarlanelabs.com

Ig Class: Mouse $IgG_{2a}$

Format: Biotin conjugated Ig buffered in PBS, 0.02% $NaN_3$ and EIA grade BSA as a stabalizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography).

Antibody Concentration: 0.1 mg/ml

**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 1.0-0.5 µg***** of **CL044B or CL044B-5**.
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 **CLCSA1001** (Streptavidin-FITC) 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:

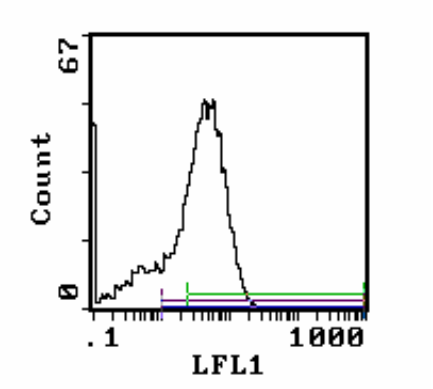
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Wistar
Cell Concentration: $1 \times 10^6$ cells per test
Antibody Concentration Used: 0.5 µg/$10^6$ cells
Isotypic Control: Biotin Mouse $IgG_{2a}$ (CLCMG2A15)

| Cell Source | Percentage of cells stained above control: |
|---|---|
| Thymus | 96.3% |
| Spleen | 66.2% |
| Lymph Node | 89.9% |




Cell Source: Spleen
Percentage of cells stained above control: 66.2%

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**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.**

**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.
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