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# TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

*Please contact CEDARLANE® for lot specific information.*

## **FITC Anti-Rat CD44 Monoclonal Antibody**

**CL044F**

**CL044F-5**

**LOT: 4431**

### **DESCRIPTION:**

Cedarlane's anti-rat CD44 monoclonal antibody recognizes an epitope on both standard CD44 and its splice variant. CD44 is expressed on most leukocytes except a sub population of B cells. Its expression is increased on T and B blasts.

This antibody is suitable for immunoprecipitation, flow cytometry and immunohistochemistry on frozen sections.

### **PRESENTATION:**

100 µg (CL044F) or 500 µg (CL044F-5) FITC conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> 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. Avoid prolonged exposure to light.

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

**CEDARLANE®**  
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**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<sub>2a</sub>

Format: FITC conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> and EIA grade BSA as a stabilizing 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<sup>®</sup>-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 1.0-0.5  $\mu$ g\* of **CL044F or CL044F-5**.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.  
(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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

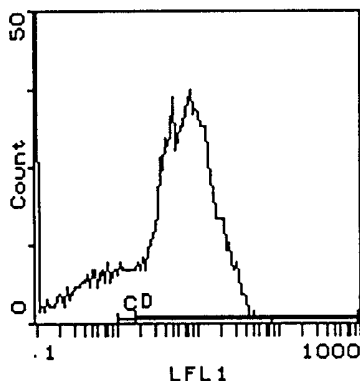
Cell Concentration:  $1 \times 10^6$  cells per tests

Antibody Concentration Used:  $1.0 \mu\text{g}/10^6$  cells

Isotypic Control: FITC Mouse IgG<sub>2a</sub> (CMG2A01)

Cell SourcePercentage of cells stained above control:

Thymus	99.6%
Spleen	75.9%
Lymph Node	96.8%



Cell Source: Spleen

Percentage of cells stained above control: 75.9%

**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:**

1. Patterson, D.J., W.A. Jefferies, J.R. Green, M.R. Brandon, P. Corthesy, M. Puklavec, and A. F. Williams. (1987) Antigens of activated rat T lymphocytes including a molecule of 50,000  $M_r$  detected only on CD4 positive T blasts. *Molec. Immunol.* 24(12): 1281-1290.
2. Arch, R., K. Wirth, M. Hofmann, H. Ponta, S. Matzku, P. Herrlich, and M. Zollen. (1992) Participation in normal immune response of a metastases inducing splice variant of CD44. *Science* 257:682-685.

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