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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 OX-2 Monoclonal Antibody**

**CL082F**  
**CL082F-3**  
**LOT: 8231**

### **DESCRIPTION:**

Cedarlane's mouse anti-rat OX-2 monoclonal antibody recognizes a monomorphic determinant present on rat thymocytes, brain, follicular dendritic cells in lymphoid organs, vascular endothelium, and at low levels on some smooth muscle and B lymphocytes. The purified brain and thymocyte OX-2 antigens are glycoproteins with apparent M.W. of 41 KDa and 47 KDa respectively. The amino acid composition of brain and thymocyte OX-2 antigen are very similar and are antigenically similar to those found on other tissues. The carbohydrate composition shows that this antigen is highly glycosylated (brain OX-2 - 24% and thymocyte OX-2 - 33% carbohydrate by weight).

The OX-2 antigen shows similarities to the Thy-1 antigen in its odd pattern of tissue distribution, carbohydrate composition and characteristic migration on SDS-PAGE. Also, the OX-2 antigens, like Thy-1 antigens, have homologies with immunoglobulin domains; the overall structure of OX-2 is similar to an Ig light chain or the T cell receptor  $\beta$  chain. Because of its distribution, it is thought to play a role in mediating recognition events at cell surfaces.

This clone can be used in flow cytometry, affinity chromatography, immunohistochemistry and in binding assays. It is also useful for labeling the follicular dendritic cells thought to be involved in the generation of B cell memory as it does not label the Ia-positive dendritic cells present in the T-dependant areas of lymphoid organs.

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

**CEDARLANE®**  
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**website: [www.cedarlanelabs.com](http://www.cedarlanelabs.com) • e-mail: [info@cedarlanelabs.com](mailto:info@cedarlanelabs.com)**

**PRESENTATION:**

100 µg (CL082F) or 300 µg (CL082F-3) 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 portions at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles. Avoid prolonged exposure to light.

**SPECIFICATIONS:**

Clone: MRC OX-2

Hybridoma Production: Spleen cells from BALB/c mice immunized with rat thymocyte membrane glycoproteins were fused with mouse NS-1 myeloma cells.

Specificity: Rat thymocytes, brain, follicular dendritic cells in lymphoid organs, vascular endothelium, and at low levels on some smooth muscle and B lymphocytes

Ig Class: Mouse IgG<sub>1</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>-R 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 µl of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 0.5-0.2 µg\* of **CL082F** or **CL082F-3** per  $10^6$  cells.
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 fluorochemicals are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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:

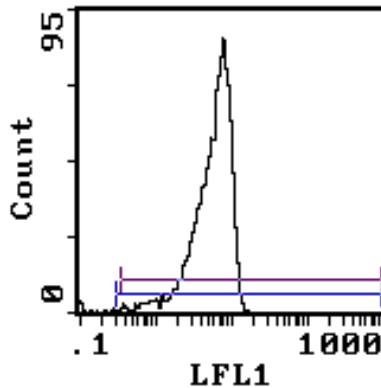
Rat Strain: Wistar

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

Antibody Concentration Used: 0.5 µg in 50 µl /  $10^6$  cells

Isotypic Control: FITC Mouse IgG<sub>1</sub> (CLCMG101)

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	98.7%
Spleen	35.7%
Lymph Node	39.9%



Cell Source: Thymus

Percentage of cells stained above control: 98.7%

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

**\* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

**REFERENCES:**

- 1) McMaster, Robert W. and Williams, Alan F. (1979) *Eur. J. Immunol.* 9:426-433. Identification of Ia glycoproteins in rat thymus and purification from rat spleen.
- 2) Barclay, A. Neil. (1981) *Immunology* 44:727-736. Different reticular elements in rat lymphoid tissues identified by localization of Ia, Thy-1 and MRC OX-2 antigens.
- 3) Barclay, A. Neil and Ward, Harry A. (1982) *Eur. J. Immunol.* 12:447-458. Purification and Chemical Characterization of Membrane Glycoproteins from Rat Thymocytes and Brain, Recognized by Monoclonal Antibody MRC OX-2.
- 4) Clark, Melanie J., Gagnon, Jean, Williams, Alan F. and Barclay, A. Neil. (1985) *EMBO Journal* 4:113-118. MRC OX-2 antigen: a lymphoid/neuronal membrane glycoprotein with a structure like a single immunoglobulin light chain.
- 5) Barclay, A. Neil. (1981) *Immunology* 42:593-600. The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues.

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