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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

PE Anti-Rat OX-2 Monoclonal Antibody

CL082PE
CL082PE-3
LOT: 8252

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 β 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...

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PRESENTATION:

50 µg (CL082PE) or 300 µg (CL082PE-3) R-PE conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at 4°C. **DO NOT FREEZE.** 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 IgG₁

Format: PE conjugated Ig buffered in PBS, 0.02% NaN₃ 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[®]-R cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2×10^7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add ~1.0-0.5 µg* of **CL082PE** or **CL082PE-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 fluochromes 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 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

Results:Tissue Distribution by Flow Cytometry Analysis:

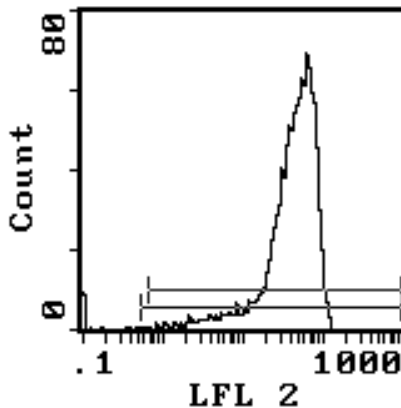
Rat Strain: Wistar

Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: 0.5 μ g in 50 μ l / 10^6 cells

Isotypic Control: PE Mouse IgG₁ (CLCMG104)

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	98.2%
Spleen	67.4%
Lymph Node	44.1%



Cell Source: Thymus

Percentage of cells stained above control: 98.2%

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.
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