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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 OX-2 Monoclonal Antibody

CL082B
CL082B-3
LOT: 8241

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...

CEDARLANE®
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or visit our website for a list of our international distributors including contact information
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PRESENTATION:

100 µg (CL082B) or 300µg (CL082B-3) 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-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_1

Format: 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. (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 **CL082B or CL082B-3.**
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 **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:

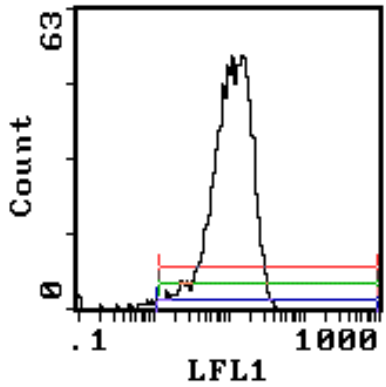
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Wistar
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 1.0 µg in 50 µl /10^6 cells
Isotypic Control: Biotin Mouse IgG_1 (CLCMG115)

Cell Source	Percentage of cells stained above control:
Thymus	97.7%
Spleen	41.2%
Lymph Node	35.5%



Cell Source: Thymus
Percentage of cells stained above control: 97.7%

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. 129*: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.

FOR RESEARCH USE ONLY

EJ/01/11/00