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# Biotin Anti-Rat SIRP (Signal-Regulatory Protein) Monoclonal Antibody

CL041B CL041B-5 LOT: 4141

## **DESCRIPTION**:

CL041B (MRC OX-41) monoclonal antibody recognizes rat SIRP (Signal-Regulatory Protein), a surface protein of about 110 kDa. SIRP is selectively expressed by myeloid cells (macrophages, monocytes, granulocytes, dendritic cells) and neurons. The SIRP antigen is a transmembrane glycoprotein with 3 immunoglobulin-like extracellular domains: an N-terminal V-set domain and two C1-set domains. The SIRP Ig domains are closely related to those of the antigen receptors, Ig, TCR and MHC. The selective expression of this antigen by myeloid and neuronal cells suggests that SIRP is involved in the modulation of myeloid and neuronal cell function.

This clone can be used in FACS analysis, immunofluorescence, immunocytochemistry, and indirect radioimmunoassays.

#### PRESENTATION:

100  $\mu$ g (**CL041B**) or 500  $\mu$ g (**CL041B-5**) Biotin 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.

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#### SPECIFICATIONS:

Clone: MRC OX-41

Hybridoma Production:

Immunization: Immunogen: Rat Peritoneal Macrophages Donor: BALB/c Spleen

Fusion Partner: NSO/U

Specificity: Rat SIRP (Signal-Regulatory Protein)

Ig Class: Mouse IgG<sub>2a</sub>

<u>Format</u>: Biotin 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  $2x10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1x10^6$  cells, representing 1 test).
- 4. To each tube, add 0.5-1.0 μg\* of **CL041B** or **CL041B-5**.
- 5. Vortex the tubes to ensure thorough mixing of antibody and cells.
- 6. Incubate the tubes for 30 minutes at  $4^{\circ}$ C.
- 7. Wash 2 times at 4°C.
- Add 100 µl of secondary antibody CLCSA1001 (Streptavidin-FITC) at 1:500 dilution.
- 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  $\mu$ l ice cold media B.
- 12. 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.

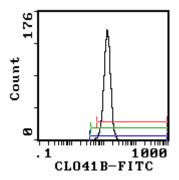
Media:

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

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: Wistar Cell Concentration :  $1 \times 10^6$  cells per tests Antibody Concentration Used:  $1.0 \ \mu\text{g}/10^6$  cells Isotypic Control: Biotin Mouse  $IgG_{2a}$ 



Cell Source: Peritoneal Macrophages 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.

#### **<u>REFERENCES</u>**:

- Robinson, A. P., White, T. M. and Mason, D. W. (1986) Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* 57: 239-247.
- Adams, S., van der Laan, L. J. W., Vernon-Wilson, E., Renardel de Lavalette, C., Dopp, E. A., Dijkstra, C. D., Simmons, D. L. and van der Berg, T. K. (1998) Signal-Regulatory Protein is Selectively Expressed by Myeloid and Neuronal Cells. *The Journal of Immunology* 161:1853-1859.

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