



# SZABO SCANDIC

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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 CD48 Monoclonal Antibody

**CL045B**  
**CL045B-5**  
**LOT: 4541**

### **DESCRIPTION:**

Cedarlane's anti-rat CD45 (Blast-1) monoclonal antibody recognizes a rat cell surface glycoprotein of 45 kDa that is present on a wide variety of hematopoietic cells and on endothelial cells. The antigen is identical to the mouse BCM1 antigen. This antibody inhibits allogeneic mixed lymphocyte reactions using lymph node cells as responders and spleen cells as stimulators. CD48 has recently been identified as a ligand of the NK cell inhibitory receptor CD244.

This antibody is suitable for flow cytometry.

### **PRESENTATION:**

100 µg (CL045B) or 500 µg (CL045B-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.

### **SPECIFICATIONS:**

Clone: MRC OX-45

Hybridoma Production:

Immunization: Immunogen: rat T cell blasts (stimulated purified  
T helper cells with allogeneic irradiated rat spleen cells)

Donor: BALB/c spleen

Fusion Partner: myeloma cell line NSO/1

Specificity: Rat CD45

Ig Class: Mouse IgG<sub>1</sub>

Format: 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 ascites fluid via Protein G Chromatography).

Antibody Concentration: 0.1 mg/ml

*Continued Overleaf...*

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

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## 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 0.2  $\mu$ g\* of **CL045B** or **CL045B-5**.
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  $\mu$ l of secondary antibody **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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:

Rat Strain: Wister

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

Antibody Concentration Used: 0.2  $\mu$ g/ $10^6$  cells

Isotypic Control: Biotin Mouse IgG<sub>1</sub> (CLCMG115)

#### Cell Source

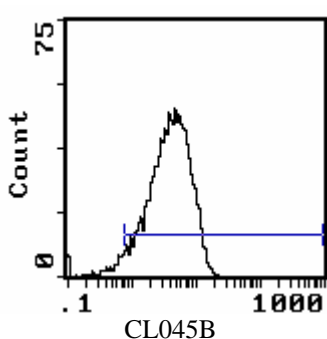
Thymus

Bone Marrow

#### Percentage of cells stained above

94.9%

98.6%



Cell Source: Thymus

Percentage of cells stained above control: 94.9%

**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. J. Arvieux, W.A. Jefferies, D. J. Patterson, A. F. Williams and J.R. Green. (1986) Monoclonal antibodies against a rat leukocyte antigen block antigen-induced T-cell responses via an effect on accessory cells. *Immunol.* **58** 337-342.
2. Wong *et al.*. (1990) Mouse BCM1 Antigen. *J Exp Med.* 171:2115.

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