



# SZABO SCANDIC

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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 Endothelium Monoclonal Antibody**

**CL043F**  
**CL043F-5**  
**LOT: 4331**

### **DESCRIPTION:**

Cedarlane's anti-rat endothelium monoclonal antibody recognizes a surface protein of MW 90 kDa and generally reacts with all vascular endothelium in the rat except that of brain capillaries (1). This is the reciprocal tissue pattern to that of the transferrin receptor. It has been shown that the expression of CL043F is on the luminal surface of blood vessels. CL043F labels all peritoneal macrophages, a sub-population of alveolar macrophages (65%) and rare interstitial cells in the brain and heart (1). In addition, Cedarlane's anti-rat endothelium mAb labels circulating erythrocytes, 22% of peripheral blood mononuclear cells and 17% of nucleated cells in bone marrow (1).

CL043F does not label granulocytes, dendritic cells, lymphocytes, or lymphocyte blasts, thymocytes, lymph node cells, mast cells and platelets. CL043F has been invaluable in the demonstration of molecular heterogeneity of vascular endothelium.

### **PRESENTATION:**

100 µg (CL043F) or 500 µg (CL043F-5) 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 portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles. Avoid prolonged exposure to light.

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

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**SPECIFICATIONS:**

Clone: MRC OX-43

**Hybridoma Production:**

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

Fusion Partner: NSO/U

Specificity: Rat Vascular Endothelium

Isotype: Mouse IgG<sub>1</sub>

Presentation: 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>-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 0.2-0.5  $\mu$ g\* of **CL043F or CL043F-5**.
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 fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
9. 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: Fischer

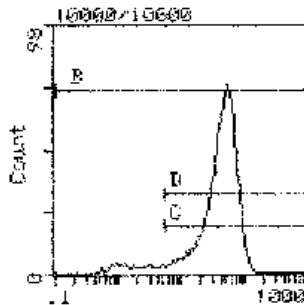
Cell Concentration :  $1 \times 10^6$  cells per test

Antibody Concentration Used: 0.5  $\mu$ g/ $10^6$  cells

Isotypic Control: FITC Mouse IgG<sub>1</sub>, $\kappa$

Cell SourcePercentage of cells stained above control:

Thymus	3.0%
Peritoneal Macrophages	92.4%

**LFL1**

Cell Source: Peritoneal Macrophages

Percentage of cells stained above control: 92.4%

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

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

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

Antibody Concentration Used: 0.5  $\mu\text{g}/10^6$  cells

Strains Tested: Wistar, Brown Norway, Buffalo, Fischer 344

Positive: Buffalo, Brown Norway, Fischer 344, Wistar

Negative: none

**REFERENCES:**

- 1) Robinson, A.P., White, T.M. and D.W. Mason. (1985) Immunology. 57, 231-237. MRC OX-43: a monoclonal antibody which reacts with all vascular endothelium in the rat except that of brain capillaries.

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