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Place your order with CEDARLANE® or your local distributor.

*Please contact CEDARLANE® for lot specific information.*

**Biotin Anti-Rat RT1.A<sup>c</sup>  
Monoclonal Antibody**

**CL027B  
CL027B-5  
LOT: 2741**

**DESCRIPTION:**

Cedarlane's anti-rat RT1.A<sup>c</sup> recognizes a polymorphic determinant of the MHC class I antigen in the rat. ("c" and "n" haplotypes). CL027B is an excellent antibody for labelling cells of donor or host origin in bone marrow chimeras. (1,2)

Applications include flow cytometry and immunohistochemistry using cryostat sections (however, cross-reactivity with Lewis rats have been shown to occur in some instances).

**PRESENTATION:**

100 µg (CL027B) or 500 µg (CL027B-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.

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

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**SPECIFICATIONS:**

Clone: MRC OX-27

Hybridoma Production:

Immunization: Immunogen: Phytohaemagglutinin Blasts

Donor: BALB/c spleen

Fusion Partner: P3-NS1-1-Ag4 (NS1/1)

Specificity: Rat RT1.A<sup>c</sup> (rat MHC Class I polymorphic)

Ig Class: Mouse IgG<sub>2a</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 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 0.5-1.0  $\mu$ g\* of **CL027B or CL027B-5** 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.
7. Wash 2 times at 4°C.
8. Add 100  $\mu$ l of secondary antibody **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution. **Not recommended for use with a PE secondary.**
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 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:

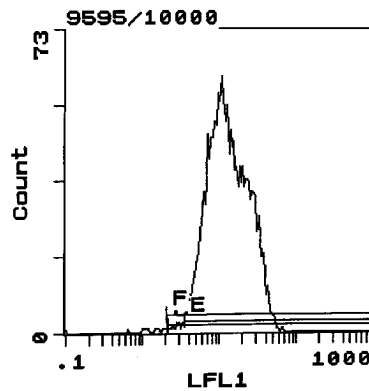
Rat Strain: Brown Norway

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

Antibody Concentration Used:  $1.0 \mu\text{g}/10^6$  cells

Isotypic Control: Biotin Mouse IgG<sub>2a</sub>

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	65.4%
Spleen	98.9%
Lymph Node	100%



Cell Source: Spleen

Percentage of cells stained above control: 98.9%

**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.**

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

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

Antibody Concentration Used:  $1.0 \mu\text{g}/10^6$  cells

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

Positive: Brown Norway

Negative: Buffalo, Wistar, Fischer, ACI

**REFERENCES:**

- 1) Butcher, G.W. (1987) 19: 3-21. Monoclonal antibodies specific for alloantigens of the rat. Rat Membrane Alloantigens News.
- 2) Jeffries, W.A. et al. (1985) J. Exp. Med. 162: 117-127. Authentic T Helper CD4 (W3/25) antigen on rat peritoneal macrophages.

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