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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

**Purified Anti-Rat RT1.A^c
Monoclonal Antibody**

**CL027AP
CL027AP-2
LOT: 2721**

DESCRIPTION:

Cedarlane's anti-rat RT1.A^c recognizes a polymorphic determinant of the MHC class I antigen in the rat ("c" and "n" haplotypes). CL027AP is an excellent antibody for labelling cells of donor or host origin in bone marrow chimeras. (1,2)

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

PRESENTATION:

250 µg (CL027AP) or 500 µg (CL027AP-2) purified Ig buffered in PBS and 0.02% sodium azide (NaN₃).

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...

CEDARLANE®
LABORATORIES LIMITED



*toll free: 1-800-268-5058
in North America*

phone: (905) 878-8891 • fax: (905) 878-7800

5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

or visit our website for a list of our international distributors including contact information
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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^c (rat MHC Class I polymorphic)

Ig Class: Mouse IgG_{2a}

Format: Purified Ig buffered in PBS and 0.02% NaN₃. (Purified from ascitic fluid via Protein G Chromatography)

Antibody Concentration: 1.0 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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.5-2.0 μ g* of **CL027AP or CL027AP-2**.
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 μ l of secondary antibody **CLCC30201** (FITC Goat anti-mouse IgG (H+L)) at 1:500 dilution. **Not recommended for use with PE secondary.**
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 μ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μ 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 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

Results:Tissue Distribution by Flow Cytometry Analysis:

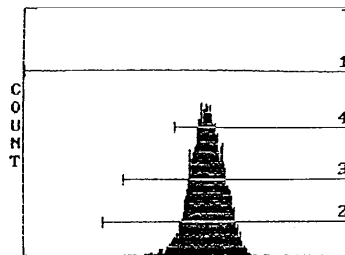
Rat Strain: Brown Norway

Cell Concentration : 1×10^6 cells per test

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

Isotypic Control: Mouse IgG_{2a}

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	39.8%
Spleen	95.9%
Lymph Node	99.9%



LFL1

Cell Source: Spleen

Percentage of cells stained above control: 95.9 %

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 : 1×10^6 cells per test

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

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

Positive: Brown Norway

Negative: Lewis, Wistar, Fischer 344 Buffalo, 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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