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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Rat RT1.D Monoclonal Antibody

**CL011AP
CL011AP-2
LOT: 1121**

DESCRIPTION:

Cedarlane's anti-rat RT1.D monoclonal antibody recognizes a monomorphic determinant on the α chain of the rat Ia antigen and appears to be the rat homologue of mouse Ia-E. It recognizes the rat Ia product present on B, but not T cells from lymph node or thoracic duct lymph. It does not bind to thymocytes or erythrocytes. The antibody does not cross-react with rat Ia-A or mouse Ia-E antigen, but rabbit antibody raised against the antibody affinity column-purified MRC OX-17 antigen cross-reacted on tissues of mice expressing Ia-E mouse antigen but not on those mouse strains that were Ia-E antigen negative (2).

PRESENTATION:

250 μ g (CL011AP) or 500 μ g (CL011AP-2) purified Ig buffered in PBS + 0.02% NaN₃

STORAGE/STABILITY:

Stable at 4°C. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

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

CEDARLANE®
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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: MRC OX-17

Hybridoma Production:

Immunization: Immunogen: Rat spleen membrane glycoproteins
depleted of Ia-A antigens.

Immunocyte Donor: BALB/c spleen

Fusion Partner: X63 Ag8.653

Specificity: Rat RT1.D (Rat Ia-E)

Isotype: Mouse IgG₁

Presentation: Purified Ig buffered in PBS with the addition of 0.02% NaN₃. (Purified from ascitic fluid via Protein G Chromatography)

Antibody Concentration: 1.0 mg/ml

FLOW CYTOMETRY ANALYSIS:

1. Prepare 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 cells to 1×10^6 cells in approximately 50 μ l media A in a microcentrifuge tube. (i.e. 50 μ l of cells resuspended to 2×10^7 cells/ml). The contents of 1 tube represent 1 test.
4. To each tube add 50 μ l of a 1:250 - 1:500 dilution of **CL011AP** or **CL011AP-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 1:700 dilution.
9. Incubate 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 phosphate buffered saline. (This stains dead cells by intercalating DNA).

MEDIA:

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

Rat Strain: Lewis Rat

Cell Concentration: 1×10^6 cells per test

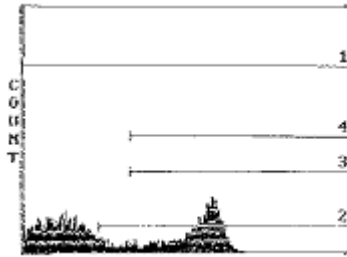
Antibody Concentration: 1:400

Isotypic Control: Mouse IgG₁, κ

CELL SOURCE

PERCENT STAINING

Thymus	12%
Spleen	34%
Lymph Node	25%



LFL1

Cell Source: Splenocytes

Percentage of Cells Stained Above Control: 34%

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:

Procedure: as above

Antibody Concentration: 1:100

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

Positive: Wistar, Buffalo, Brown Norway, ACI, Fischer 344

Negative: none

REFERENCES:

1. Kearney, J.F., Radbruch, A., Leisegang, B. and K. Rajewsky. (1979) A New mouse myeloma cell line that has lost Immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* 123, 1548-1550.
2. Fukumoto, T., McMaster, W.R. and A.F. Williams. (1982) Mouse monoclonal antibodies against rat major histocompatibility antigens. Two Ia antigens and expression of Ia and Class I antigens in rat thymus. *Eur. J. Immunol.* 12, 237-243.
3. Barclay, A.N. (1981) Different reticular elements in rat lymphoid tissue identified by localization of Ia, thy-1 and MRC OX-2 antigens. *Immunology.* 42, 593-600.

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