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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 $\alpha\beta$ TCR Monoclonal Antibody

CL057AP
CL057AP-2
LOT: 5721

DESCRIPTION:

Cedarlane's anti-rat $\alpha\beta$ T cell receptor monoclonal antibody detects a T cell-specific heterodimeric 40 and 46 kD cell surface protein that is expressed on the vast majority, but not all, peripheral rat T cells. CL057AP induces blastoid transformation and IL-2R expression in T-cells when cross linked on an artificial surface. It also induces cell proliferation when exogenous IL-2 is added.

Applications: Immunoprecipitation, flow cytometry, immunohistochemical staining on frozen sections.

PRESENTATION:

250 μg (CL057AP) or 500 μg (CL057AP-2) purified Ig buffered in PBS, 0.02% NaN_3 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 repeated freeze/thaw cycles.

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

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

SPECIFICATIONS:

Clone: R73

Hybridoma Production:

Immunization: Immunogen: Rat T Blasts
Donor: BALB/c Spleen

Fusion Partner: X63-Ag 8.653

Specificity: Rat $\alpha\beta$ TCR

Ig Class: Mouse IgG₁

Format: Purified Ig buffered in PBS, 0.02% NaN₃ (Purified from BioReactor Supernatant via Protein G Affinity 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 0.2 - 0.5 μ g* of **CL057AP or CL057AP-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.
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 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: Fischer

Cell Concentration : 1×10^6 cells per test

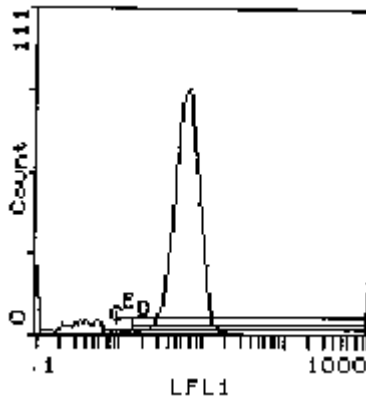
Antibody Concentration Used: $0.2 \mu\text{g}/10^6$ cells

Isotypic Control: Mouse IgG₁

Cell SourcePercentage of cells stained above control:

Thymus	61.1%
Splenic T Cells*	88.8%

*(T cells isolated with CL102 - Cedarlane's Rat T Cell Recovery Column Kit)



Cell Source: Splenic T-cells

Percentage of cells stained above control: 88.8%

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

Cell Concentration : 1×10^6 cells per test

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

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

Positive: Buffalo, Wistar, Fischer 344, Brown Norway

Negative: none

REFERENCES:

1. Hunig, T., Wallny, H.J., Hartley, J.K., Lawetzky, A., and Tiefenthaler, G. (1989). A Monoclonal Antibody to a Constant Determinant of the Rat T-Cell Antigen Receptor that Induces T-Cell Activation. *J. Exp. Med.* 169:73-86.

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