



# SZABO SCANDIC

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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  $\mu\text{g}$  (CL057AP) or 500  $\mu\text{g}$  (CL057AP-2) purified Ig buffered in PBS, 0.02%  $\text{NaN}_3$  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...

**CEDARLANE®**  
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**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 IgG<sub>1</sub>

Format: Purified Ig buffered in PBS, 0.02% NaN<sub>3</sub> (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  $2 \times 10^7$  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 **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  $\mu$ 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  $\mu$ 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: Fischer

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

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

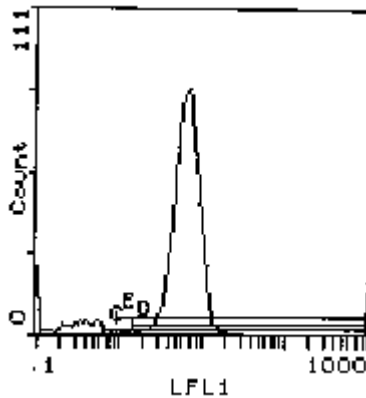
Isotypic Control: Mouse IgG<sub>1</sub>

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 :  $1 \times 10^6$  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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