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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 CD28 Monoclonal Antibody

CL028AP
CL028AP-2
LOT: 2821

DESCRIPTION:

Cedarlane's anti-rat CD28 monoclonal antibody recognizes a 90kDa homeodimeric cell surface glycoprotein. CD28 has been found to be a potent costimulatory receptor on T cells. It is expressed on all peripheral rat αβ and most γδ T cells, as well as on approximately half of all NK cells.

This clone can costimulate T cell proliferation and IL-2 secretion by resting rat T cells.

Applications: Flow cytometry, Immunoprecipitation

PRESENTATION:

250 µg (CL028AP) or 500 µg (CL028AP-2) purified Ig buffered in PBS and 0.02% NaN_3

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

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or visit our website for a list of our international distributors including contact information

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

SPECIFICATIONS:

Clone: JJ319

Hybridoma Production:

Immunization: Immunogen: Rat CD28 transfected A20J
Donor: BALB/c spleen

Fusion Partner: X63-Ag8.653

Specificity: Rat CD28

Ig Class: Mouse IgG_1

Format: Purified Ig buffered in PBS and 0.02% NaN_3. (Purified from bioreactor material 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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 1.0 µg* of **CL028AP or CL028AP-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 µl of secondary antibody **CLCC30204** (PE Goat anti-mouse IgG (H+L)) at 1:50 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 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl 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:

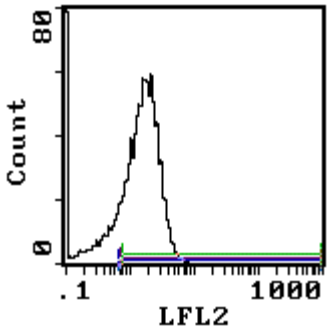
Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Fischer
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 1.0 µg/10^6 cells
Isotypic Control: Mouse IgG_1 (CMG100)

Cell Source	Percentage of cells stained above control:
Thymus	18.0%
Splenic T Cells*	71.7%

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

Cell Source: Splenic T Cells



Percentage of cells stained above control: 71.7%

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.**

REFERENCES:

1. Tacke, M., Georgina J. Clark, Margaret J. Dallman and Thomas Hünig. (1995). Cellular Distribution and Costimulatory Function of Rat CD28. J. Immunol. 154:5121-5127

FOR RESEARCH USE ONLY

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