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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Rat CD28 Monoclonal Antibody

CL028B
CL028B-5
LOT: 2841

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

PRESENTATION:

100 µg (CL028B) or 500 µg (CL028B-5) Biotin conjugated 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 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: JJ319

Hybridoma Production:

Immunization: Immunogen: Rat CD28 transfected A20J cells
Donor: BALB/c spleen
Fusion Partner: X63-Ag 8.653

Specificity: Rat CD28

Ig Class: Mouse IgG_1

Format: Biotin conjugated 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. (Purified from Bioreactor supernatant via Protein G Chromatography)

Antibody Concentration: 0.1 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 1×10^6 cells, representing 1 test).
4. To each tube, add 1.0 µg***** of **CL028B or CL028B-5** per 10^6 cells.
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 **CLCSA1004** (Streptavidin-PE) at a 1:50 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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).

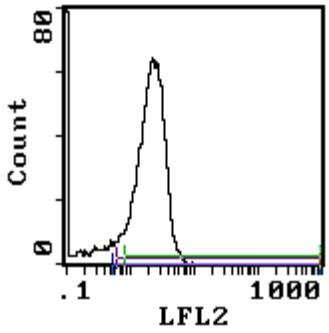
Results:

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: Biotin Mouse IgG_1

Cell Source	Percentage of cells stained above control:
Thymus	25.4%
Splenic T Cells*	73.2%

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



Cell Source: Splenic T Cells
Percentage of cells stained above control: 73.2%

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, Margeret 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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