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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC Anti-Rat CD28 Monoclonal Antibody

CL028F
CL028F-5
LOT: 2831

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 $\alpha\beta$ and most $\gamma\delta$ 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 (CL028F) or 500 μg (CL028F-5) FITC 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. Avoid prolonged exposure to light.

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

CEDARLANE®
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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 IgG₁

Format: FITC conjugated Ig buffered in PBS, 0.02% NaN₃ 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 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 1.0 μ g* of **CL028F** or **CL028F-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.
(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 μ l ice cold media B.
9. 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

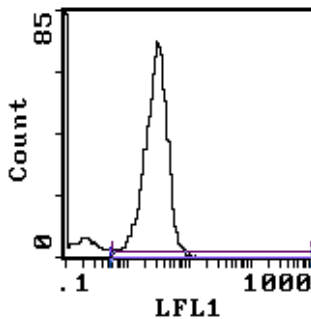
Antibody Concentration Used: $1.0 \mu\text{g}/10^6$ cells

Isotypic Control: FITC Mouse IgG₁

Cell SourcePercentage of cells stained above control:

Thymus	36.3%
Splenic T Cells*	79.7 %

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



Cell Source: Splenic T cells

Percentage of cells stained above control: 79.7%

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

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SV/02/22/99