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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 $\gamma\delta$ TCR Monoclonal Antibody

CL056B

CL056B-5

LOT: 5641

DESCRIPTION:

Cedarlane's anti-rat $\gamma\delta$ T cell receptor monoclonal antibody detects a T cell-specific heterodimeric 48 and 50 kD cell surface protein that is expressed on greater than 90% of CD3⁺ $\alpha\beta$ TCR— rat peripheral T lymphocytes, and identifies a dense network of dendritic cells in the epidermis as $\gamma\delta$ T cells. Immobilized this antibody induces a strong proliferative response in $\gamma\delta$ T cell cultures supplemented with either IL-2 or IL-4.

Applications: Flow cytometry, immunohistochemical staining on frozen sections.

PRESENTATION:

100 μ g (CL056B) or 500 μ g (CL056B-5) Biotin 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.

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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SPECIFICATIONS:

Clone: V65

Hybridoma Production:

Immunization: Immunogen: Rat T Blasts

Donor: BALB/c spleen

Fusion Partner: X63-Ag 8.653

Specificity: Rat $\gamma\delta$ TCR

Ig Class: Mouse IgG₁

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 0.1-0.2 μ g* of **CL056B or CL056B-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 μ l of secondary antibody **CLCSA1004** (Streptavidin-PE) at a 1:20 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 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

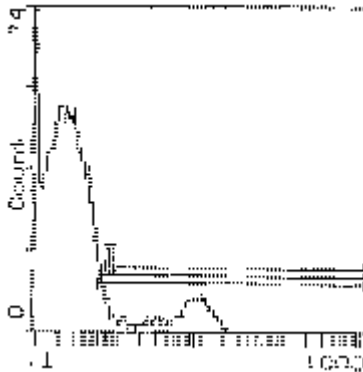
Antibody Concentration Used: 0.2 µg/ 10^6 cells

Isotypic Control: Biotin Mouse IgG₁

Cell SourcePercentage of cells stained above control:

Thymus	3.6%
Splenic T Cells*	5.3%

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



LFL2

Cell Source: Splenic T Cells

Percentage of cells stained above control: 5.3%

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. Kühnlein, P., Park, H-J., Herrmann, T., Elbe, A., and Hünig, T. (1994). Identification and Characterization of Rat $\gamma\delta$ T-Lymphocytes in Peripheral Lymphoid Organs, Small Intestine, and Skin With a Monoclonal Antibody to a Constant Determinant of the $\gamma\delta$ T cell Receptor. *J. Immunol.* 153:979-986.

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