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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Mouse CD49d Monoclonal Antibody

CL8919B
CL8919B-3
LOT:1941

DESCRIPTION:

Cedarlane's anti-mouse CD49d monoclonal antibody reacts with $\alpha 4$ integrin, which helps to mediate cell-cell and cell-matrix interactions.

$\alpha 4$ integrin combines with $\beta 1$ and $\beta 7$ integrin to form VLA-4 and LPAM-1 (Peyers patch homing receptor) respectively. VLA-4 is expressed on most peripheral lymphocytes, thymocytes and monocytes. LPAM-1 is found on peripheral lymphocytes, but few thymocytes. Fibronectin and VCAM-1 act as ligands for both VLA-4 and LPAM-1. LPAM-1 also binds the mucosal vascular addressin MAdCAM-1. (1)

Applications of this clone include flow cytometry, immunoprecipitation and immunohistochemistry. (1,2,3)

PRESENTATION:

100 μ g (CL8919B) or 300 μ g (CL8919B-3) 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...

CEDARLANE®
LABORATORIES LIMITED



toll free: 1-800-268-5058
in North America

phone: (905) 878-8891 • fax: (905) 878-7800

5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

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: R1-2

Hybridoma Production:

Immunization: Immunogen: Peyers Patch HEV binding
lymphoma line (TK1)
Donor: Fisher Spleen

Fusion Partner: P3x63Ag8.653

Specificity: Mouse CD49d ($\alpha 4$ integrin)

Ig Class: Rat IgG_{2b}

Format: 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. (Purified from ascitic fluid 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[®]-M cell separation medium (CL5030).
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 $\sim 1.0 \mu\text{g}^*$ of **CL8919B** or **CL8919B-3** 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 **CLCSA1001** (Streptavidin-FITC) at a 1:500 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:

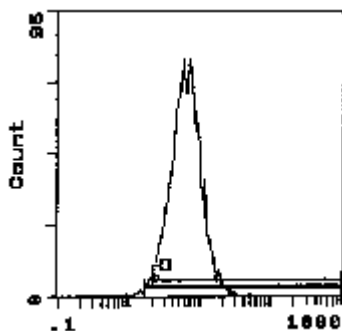
Mouse Strain: BALB/c

Cell Concentration : 1×10^6 cells per tests

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

Isotypic Control: Biotin Rat IgG_{2b}

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
TK1 Cells	97.9%
Thymus	80.0%
Spleen	85.1%
Bone Marrow	72.4%



FL1 LOG

Cell Source: TK1 Cell Line

Percentage of cells stained above control: 97.9%

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) Berlin, C., E. L. Berg, M. J. Briskin, D. P. Andrew, P. J. Kilshaw, B. Holzmann, I. L. Weissman, A. Hamann, E.C. Butcher 1993. $\alpha 4\beta 7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCam-1. *Cell* 704:185-195
- 2) Holzmann, B., I. L. Weissman 1989. Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4 like α chain associated with either of two integrin β chains, one of which is novel. *EMBO* 8:1736-1741
- 3) Holzmann, B., B. W. McIntyre, I. W. Weissman 1989. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an α chain homologous to human VLA-4 α . *Cell* 56:37-46

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