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Monoclonal Antibody to CD49d / ITGA4 - FITC

Alternate names: CD49 antigen-like family member D, Integrin alpha-4, Integrin alpha-IV, VLA-4, VLA4

Catalog No.: CL030F

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Background: Integrin alpha 4 (also called CD49d) is a 150 kDa protein that possesses a large extracellular domain involved in ligand binding, a single transmembrane domain, and an intracellular regulatory domain possessing multiple sites for phosphorylation. Integrin alpha 4 forms heterodimers with integrins beta 1 and beta 7. Integrin alpha 4 is expressed on leukocytes and leukocyte precursors, neural crest cells, and developing skeletal muscles and is essential for embryogenesis, hematopoiesis, and immune responses. The presence of integrin alpha 4 promotes cell migration and inhibits cell spreading and contractility. Integrin alpha 4 function has been implicated in the pathogenesis of multiple diseases including asthma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, hepatitis C, and multiple sclerosis, and therefore, modulation of integrin alpha 4 function has become an important target for drug discovery.

Uniprot ID: Q00651

NCBI: NP_034706.3

GeneID: 16401

Host / Isotype: Rat / IgG2b

Clone: R1-2

Immunogen: Peyers Patch HEV binding lymphoma line (TK1)

Format:
State: Liquid
Purification: Purified from ascitic fluid via Protein G Chromatography
Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.
Label: FITC

Applications:
Flow cytometry (see protocol).
Immunoprecipitation.
Immunohistochemistry. (1,2,3)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
Antibody CL030FX reacts with a4 integrin (mouse CD49d), which helps to mediate cell-cell and cell-matrix interactions.
Species: mouse.
Other species not tested.

Storage: Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid prolonged exposure to light. Shelf life: one year from despatch.

General 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. a4b7 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 a chain associated with either of two integrin b 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 a chain homologous to human VLA-4a. Cell 56:37-46

Protocols:

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.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x107 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 106 cells, representing 1 test).
4. To each tube, add 1.0 µg* of CL030F per 106 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 flurochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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).

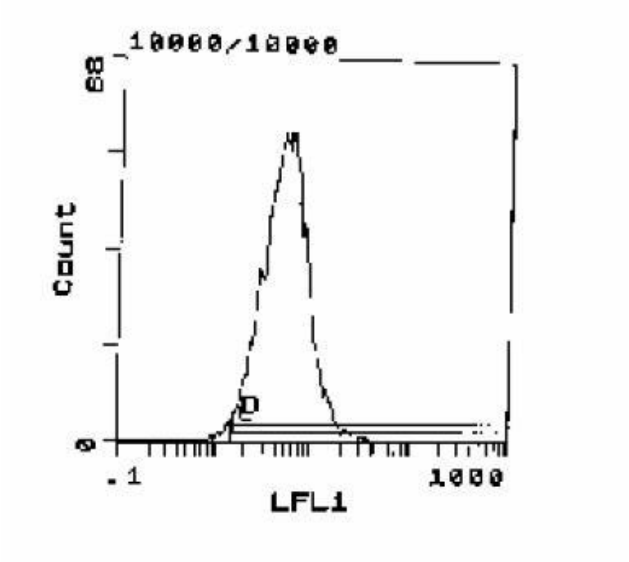
Results:
Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain: BALB/c
Cell Concentration: 1x106 cells per tests
Antibody Concentration Used: 1.0 µg/106 cells
Isotypic Control: FITC Rat IgG2b

Cell Source Percentage of cells stained above control:
TK1 cell line 96.8%
Thymus 45.6%
Spleen 88.0%
Bone Marrow 84.7%

Strain Distribution by Flow Cytometry Analysis:
Cell Concentration: 1x106 cells per tests

Antibody Concentration Used:1.0 µg /106 cells
Strains Tested: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Positive: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Negative: non

Pictures:



Cell Source: TK1 cell line
Percentage of cells stained above control: 96.8%