# Anti-Human CD49d (ALC1/1)



	REF	Σ	$\Diamond$	[A]	
PURE	49DPU	l mg	10 μL/test	1 mg/ml	
FITC	49DF-100T	100 test	20 μL/test	0,05 mg/ml	
PE	49DPE-100T	100 test	20 μL/test	0,05 mg/ml	RUO
PerCP	49DPP-100T	100 test	5 µL/test	O,2 mg/ml	
APC	49DA-100T	100 test	20 μL/test	0,2 mg/ml	

#### PRODUCT DESCRIPTION

Clone: ALC1/1;

Isotype: IqG1;

Tested application: flow cytometry;

Immunogen: The anti-CD49d monoclonal antibody derives from U-937 cell line;

Species reactivity: Human;

Storage instruction: store in the dark at 2-8 °C;

Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN $_3$ );

Recommended usage: Immunostep's CD49d, clone ALCI/I is a monoclonal antibody intended for the identification and enumeration of T and B lymphocytes and weakly on monocytes using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using I test for IO<sup>6</sup> cells;

Presentation: liquid;

Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;

Purification: Affinity chromatography;

Other names: Alpha-4 integrin chain; VLA-4 alpha chain, CD49 antigen-like family member D, Integrin alpha-IV, VLA-4 subunit alpha;

Gene ID: 3676;

Molecular weight: 150 kDa.

# 2. ANTIGEN DETAILS

Large description: Anti-CD49d (Anti-VLA- $\alpha$ -4) clone ALC/I recognizes the  $\alpha$ -chain of very-late antigen (VLA)-4, a member of the integrin family of cell adhesion molecules. VLA-4, like other integrins, is a noncovalently associated heterodimeric glycoprotein composed of  $\alpha$  and  $\beta$  subunits and is involved in cell–cell and cell–extracellular matrix interactions. The  $\beta$ -chain of the VLA-4 complex is the CD29 antigen.

The CD49d antigen binds to CS-1, an alternatively spliced domain of fibronectin. When functioning as a cell receptor, the CD49d antigen binds to the vascular cell-adhesion molecule-1 (VCAM-1). The interaction between the CD49d antigen and VCAM-1 is known to play an important role in stabilizing the adhesion of lymphocytes to endothelial cells and in mediating B-lymphocyte precursor/bone marrow stromal cell adhesion. The CD49d antigen, when associated with the ß integrin, forms a lymphocyte homing receptor for Peyer's patch, binding to the mucosal vascular addressin MAdCAM-1. The CD49d antigen is also involved in CD3-dependent CD4+ T-lymphocyte activation via its interaction with fibronectin. (16)

## WARRANTY

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Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

## . ADDITIONAL INFORMATION

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#### PROTOCOL

#### Direct Immunofluorescence Cell Surface Staining Protocol

- 1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene
- Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
- 3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
- After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
- Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
- 6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
- 7. After removing the supernatant and resuspending the cell pellet, add 300  $\mu$ L of PBS and adquire on the flow cytometer are recorded.
- 8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

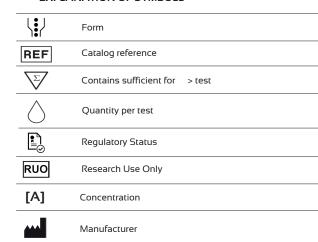
#### Indirect Immunofluorescence Cell Surface Staining Protocol

- 1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube
- Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
- Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
- Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
- Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for I5 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
- 6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
- 7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
- After removing the supernatant and resuspending the cell pellet, add 300 μL of PBS and adquire on the flow cytometer are recorded.
- 9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

#### 6. REFERENCES

- Holzmann B, McIntyre BW, Weissman IL. Identification of a murine Peyer's patch--specific lymphocyte homing receptor as an integrin molecule with an alpha chain homologous to human VLA-4 alpha. Celli 989 Jan 13:56(1):37-46.
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## 7. EXPLANATION OF SYMBOLS



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