Anti-Human CD49d (ALC1/1)

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

1. PRODUCT DESCRIPTION

Clone: ALC1/1;
Isotype: IgG1;
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 ALC1/1 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 1 test for 10^6 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-α-4) clone ALC/1 recognizes the α-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 α and ß subunits and is involved in cell–cell and cell–extracellular matrix interactions. The ß-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.[1-6]

3. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties.

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

4. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

5. PROTOCOL

Direct Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. 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.
4. 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).
5. 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 µL 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
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. 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.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 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.
8. 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

1. 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. Cell1989 Jan 13;56(1):37-46.
2. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. FASEB J1990 Aug;4(11):2868-80.
3. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell1990 Feb 23;60(4):577-84.
4. Nojima Y, Humphries MJ, Mould AP, Komoriya A, Yamada KM, Schlossman SF, et al. VLA-4 mediates CD3-dependent CD4+ T cell activation via the CS1 alternatively spliced domain of fibronectin. J Exp Med1990 Oct 01;172(4):1185-92.
5. Springer TA. Adhesion receptors of the immune system. Nature1990 Aug 02;346(6283):425-34.
6. Dittel BN, McCarthy JB, Wayner EA, LeBien TW. Regulation of human B-cell precursor adhesion to bone marrow stromal cells by cytokines that exert opposing effects on the expression of vascular cell adhesion molecule-1 (VCAM-1). Blood1993 May 01;81(9):2272-82.
7. Modderman PW. New clusters: CD29/CDw49, CD47 CD51, CD55, and CD61. In: Knapp W, Dörken B, Gilks WR, et al, eds. Leucocyte Typing IV: White Cell Differentiation Antigens. New York, NY: Oxford University Press; 1989:1017-1019.

7. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for > test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
[A]	Concentration
	Manufacturer

8. MANUFACTURED BY:

IMMUNOSTEP S.L.
Address: Avda. Universidad de Coimbra, s/n
Cancer Research Center (C.I.C)
Campus de Unamuno
37007 Salamanca (Spain)
Telf./fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com