Anti-Human CD146 (TEA1/34)

	REF	Σ		[A]	
PURE	146PU	1 mg	10 µg/test	1 mg/ml	
PURE	146PU-01MG	100 µg	10 µg/test	1 mg/ml	
FITC	146F-100T	100 test	20 µL/test	0,05 mg/ml	RUO
PE	146PE-100T	100 test	20 µL/test	0,05 mg/ml	
APC	146A-100T	100 test	20 µL/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: TEA1/34;
Isotype: IgG2a;
Tested application: flow cytometry;
Immunogen: The anti-CD146 monoclonal antibody derives from T cells from human endothelial cells HUVECs;
Species reactivity: Human;
Storage instruction: store in the dark at 2-8 °C. It does not affect purified.
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN_3);
Recommended usage: Immunostep's CD146, clone TEA1/34, is a monoclonal antibody intended for the identification and enumeration of MUC18/MCAM antigen, an activation antigen of human T lymphocytes 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: S- Endo, Cell surface glycoprotein MUC20;
Gene ID: 4162;
Molecular weight: 118 kDa.

2. ANTIGEN DETAILS

Large description: This antibody reacts with the CD146 antigen, which is expressed on melanoma cells, epithelial cells, endothelial cells, fibroblasts, activated T cells, multipotent mesenchymal stromal cells, and activated keratinocytes. CD146 mediates heterophilic cell adhesion and regulates monocyte transendothelial migration. The ligand of CD146 remains to be identified. Among peripheral whole blood leucocytes, CD146 expression is not detected, except on a subset of activated T-lymphocytes.[1-4]

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.

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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. Pickl WF, Majdic O, Fischer GF, Petzelbauer P, Fae I, Waclavicek M, et al. MUC18/MCAM (CD146), an activation antigen of human T lymphocytes. J Immunol1997 Mar 01;158(5):2107-15.
2. Anfosso F, Bardin N, Frances V, Vivier E, Camoin-Jau L, Sampol J, et al. Activation of human endothelial cells via S-endo-1 antigen (CD146) stimulates the tyrosine phosphorylation of focal adhesion kinase p125(FAK). J Biol Chem1998 Oct 09;273(41):26852-6.
3. Bardin N, Anfosso F, Masse JM, Cramer E, Sabatier F, Le Bivic A, et al. Identification of CD146 as a component of the endothelial junction involved in the control of cell-cell cohesion. Blood2001 Dec 15;98(13):3677-84.
4. Bidlingmaier S, He J, Wang Y, An F, Feng J, Barbone D, et al. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. Cancer Res2009 Feb 15;69(4):1570-7.

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:

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