# Anti-Human CD326 (VU-1D9) (EpCAM)



\∎/	REF	T	$\bigcirc$	[A]	
PURE	326PU	l mg	l µg/test	1 mg/ml	
PURE	326PU-01MG	100 µg	l µg/test	1 mg/ml	RUO
APC	326A-100T	100 test	20 µL/test	0,05 mg/ml	

# 1. PRODUCT DESCRIPTION

- Clone: VU-1D9;
- Isotype: Mouse IgG1, kappa;
- Tested application: flow cytometry;

Immunogen: The anti-monoclonal antibody derives from Human small cell lung carcinoma cell line NCI-H69; 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\_);
- Recommended usage: Immunostep's CD326, clone VU-1D9 is a monoclonal antibody intended for:
- Flow cytometry immunophenotyping: identification and enumeration of EpCAM antigen. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10<sup>6</sup> cells.
- Exosomes detection: these products are effective for detection of exosomes in combination with #ExoStep Kit and #capture beads. For this application it will could be necessary to assay with different quantities;

#### Presentation: liquid;

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

Other names: Ep-CAM, tumor associated calcium signal transducer 1, epithelial cell surface antigen, epithelial glycoprotein 2, EGP2, adenocarcinoma associated antigen; Gene ID: 4072;

Molecular weight: 40 kDa.

## 2. ANTIGEN DETAILS

**Large description**: CD326 is a protein highly expressed in bone marrow, colon, lung, and most normal epithelial cells. It is expressed on carcinomas of gastrointestinal origin. Recently, it has been reported that CD326 expression occurs during the early steps of erythrogenesis. CD326 antigen is an immunotherapeutic target for the treatment of human carcinomas.<sup>(1, 2)</sup>

## 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.

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PROTOCOL

5.

#### 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.
- Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
- 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 I,5 ml of an erythrocyte-lysing solution and 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.
- After removing the supernatant and resuspending the cell pellet, add 300 μL of PBS and adquire on the flow cytometer are recorded.
- 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.
- 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.
- 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 I,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  $\mu L$  of PBS and adquire on the flow cytometer are recorded.
- 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

- Munz M, Kieu C, Mack B, Schmitt B, Zeidler R, Gires O. The carcinoma-associated antigen EpCAM upregulates c-myc and induces cell proliferation. Oncogene2004 Jul 29;23(34):5748-58.
- Rao CG, Chianese D, Doyle GV, Miller MC, Russell T, Sanders RA, Jr., et al. Expression of epithelial cell adhesion molecule in carcinoma cells present in blood and primary and metastatic tumors. Int J Oncol2005 Jul;27(I):49-57.

## 7. EXPLANATION OF SYMBOLS

L J	Form		
REF	Catalog reference		
$\sum$	Contains sufficient for > test		
$\bigcirc$	Quantity per test		
	Regulatory Status		
RUO	Research Use Only		
[A]	Concentration		
	Manufacturer		

MANUFACTURED BY:

8.

#### Y: IMMUNOSTEP S.L.



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