Anti-Human CD28 (CD28.2)

















FITC

28F-100T

20 µL/test 2 mg/ml 100 test



1. PRODUCT DESCRIPTION

Clone: CD28.2;

Isotype: IaG1:

Tested application: flow cytometry;

Immunogen: The anti-CD28 monoclonal antibody derives from human CD28 Transfected

Species reactivity: Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macague, Squirrel Monkey, Capuchin Monkey, Sooty Mangabey;

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

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

Recommended usage: Immunostep's CD28, clone CD28,2, is a monoclonal antibody intended for the identification and enumeration of human T cells subsets using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using I test for 106 cells;

Presentation: liquid;

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

Purification: Affinity chromatography:

Other names: T-cell-specific surface glycoprotein CD28, TP44;

Gene ID: 940:

Molecular weight: 44 kDa.

2. ANTIGEN DETAILS

Large description: CD28 is expressed on approximately 29 % of CD4+. CD28 mediates cell adhesion trough the two ligands, CD80, expressed on activated B cells, Cross-blooking of CD28 induces T cell activation, suggesting an important role for CD28 in the interaction between B and T cells. This antibody recognizes an homodimeric transmembrane glycoprotein present on most mature T cells, thymocytes and plasma cells.(1-5)

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.

ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

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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.

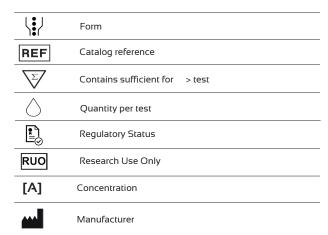
Indirect Immunofluorescence Cell Surface Staining Protocol

- 1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test
- 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 uL 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

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7. **EXPLANATION OF SYMBOLS**



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