Anti-Human CD28 (CD28.2)

	REF	Σ		[A]	
FITC	28F-100T	100 test	20 µL/test	2 mg/ml	RUO

1. PRODUCT DESCRIPTION

Clone: CD28.2;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-CD28 monoclonal antibody derives from human CD28 Transfected Cell Line;
Species reactivity: Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, 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 (NaN_3);
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 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: 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.

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. Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc Natl Acad Sci U S A1987 Dec;84(23):8573-7.
2. Freeman GJ, Gribben JG, Boussiotis VA, Ng JW, Restivo VA, Jr., Lombard LA, et al. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. Science1993 Nov 5;262(5135):909-11.
3. June CH, Ledbetter JA, Linsley PS, Thompson CB. Role of the CD28 receptor in T-cell activation. Immunol Today1990 Jun;11(6):211-6.
4. Ledbetter JA, Martin PJ, Spooner CE, Wofsy D, Tsu TT, Beatty PG, et al. Antibodies to Tp67 and Tp44 augment and sustain proliferative responses of activated T cells. J Immunol1985 Oct;135(4):2331-6.
5. Moretta A, Pantaleo G, Lopez-Botet M, Moretta L. Involvement of T44 molecules in an antigen-independent pathway of T cell activation. Analysis of the correlations to the T cell antigen-receptor complex. J Exp Med1985 Sep 1;162(3):823-38.

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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