Anti-Human CD25 (TP1/6)

	REF	Σ		[A]	
PURE	25PU	1 mg	1 mg/ml	10 mg/ml	RUO
APC	25A-100T	100 test	20 µL/test	0,05 mg/ml	
CF-Blue	25CFB-100T	100 test	5 µL/test	0,2 mg/ml	
PE	25PE-X-100T	100 test	20 µL/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: TP1/6;
Isotype: IgG2b;
Tested application: flow cytometry;
Immunogen: The anti-CD25 monoclonal antibody derives from PMA and Ionomycin activated T cells blast (Human);
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 CD25, clone TP1/6 is a monoclonal antibody intended for the identification and enumeration of activated T cells and B cells, monocytes/macrophages, T reg. 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: Low affinity IL-2R, IL-2R α chain, Tac, p55, Interleukin-2 receptor subunit alpha, IL-2 receptor subunit alpha, IL-2-RA, IL-2R subunit alpha, IL2-RA, TAC antigen;
Gene ID: 3559;
Molecular weight: 55 kDa.

2. ANTIGEN DETAILS

Large description: CD25 recognizes a type I transmembrane glycoprotein. CD25 is an epitope B of the IL-2 alpha receptor. Expression of human CD25 (the alpha subunit of the IL-2 receptor complex) is dramatically upregulated on activated T cells, B cells, NK cells and macrophages.[1-3]

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 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 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. Chung IY, Dong HF, Zhang X, Hassanein NM, Howard OM, Oppenheim JJ, et al. Effects of IL-7 and dexamethasone: induction of CD25, the high affinity IL-2 receptor, on human CD4+ cells. Cell Immunol2004 Nov-Dec;232(1-2):57-63.

2. Leonard WJ, Depper JM, Robb RJ, Waldmann TA, Greene WC. Characterization of the human receptor for T-cell growth factor. Proc Natl Acad Sci U S A1983 Nov;80(22):6957-61.

3. Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. J Immunol1981 Apr;126(4):1393-7.

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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