Anti-Human CD3/CD4/CD45 (33-2A3/HP2/6/D3/9)

	REF		
FITC/PE/PerCP	3F4PE45PP1-50T	50 test	RUO

1. PRODUCT DESCRIPTION

Clones: 33-2A3, H2/6, D3/9
Isotype: IgG2a, IgG2a, IgG1
Tested application: flow cytometry
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 CD3/CD4/CD45, is a monoclonal antibody intended for simultaneous detection and enumeration of lymphocytes. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 106 cells.
Presentation: liquid
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma.
Purification: Affinity chromatography.

2. ANTIGEN DETAILS

Large description: The CD3 monoclonal antibody is directed against the CD3- antigen (T3- antigen), which is expressed on human T lymphocytes. The monoclonal antibody reacts with 80-90% human peripheral T lymphocytes and medullary thymocytes. The monoclonal antibody does not react with B-cells, monocytes, granulocytes and platelets. The monoclonal antibody is mitogenic for resting T lymphocytes and it blocks the cytolytic activity of CTL clones.

The CD4 monoclonal antibody is directed against the CD4-antigen (T4-antigen),which is expressed on human peripheral T lymphocytes and 80% of thymocytes. The monoclonal antibody reacts on a low level with human monocytes and macrophages. The monoclonal antibody does not react with B-cells ,granulocytes and thrombocytes.

The CD45 monoclonal antibody is directed against the CD45- antigen, defined T200 or Leucocyte Common Antigen. The antibody reacts with all cells of the haemopoietic lineage, not with cells of other lineages.

Please, refer to www.immunostep.com technical support for more information.

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.

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 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.

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. Tunnacliffe A, Olsson C, Traunecker A, Krissansen GW, Karjalainen K, de la Hera A. T3.2. The majority of CD3 epitopes are conferred by the epsilon chain. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leucocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 295-6.

2. Schmidt RE. M6. CD16 cluster workshop report. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, et al., editors. Leucocyte typing V. White cell differentiation antigens. Proceedings of the 5th International Workshop and Conference; 1993 Nov 3-7; Boston, USA. Oxford, New York, Tokyo: Oxford University Press; 1995. Volume 2. p. 805-6.

3. Tamm A, Schmidt RE. The binding epitope of human CD16 (FcgRIII) monoclonal antibodies. Implications for ligand binding. J Immunol 1996;157:1576-81.

7. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

8. MANUFACTURED BY:

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