Anti-Human CD45 (D3/9)

	REF	Σ		[A]	
PURE	45PU1	1 mg	10 µL/test	1 mg/ml	RUO
CF-Blue	45CFB1-100T	100 test	5 µL/test	0,2 mg/ml	
PerCP	45PP1-100T-X	100 test	20 µL/test	0,2mg/ml	

1. PRODUCT DESCRIPTION

Clone: D3/9;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-CD45 monoclonal antibody derives from T cells from leukemic HPB-ALL;
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 CD45, clone D3/9 is a monoclonal antibody intended for the identification and enumeration of T200 protein 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: Receptor-type tyrosine-protein phosphatase C, Leukocyte common antigen, L-CA, T200;
Gene ID: 5788;
Molecular weight: 180, 195, 205, 220 and 240 kDa.

2. ANTIGEN DETAILS

Large description: The 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.

CD45 plays a critical role in T and B cell antigen receptor-mediated activation by dephosphorylating substrates including p56Lck, p59Fyn, and other Src family kinases and associated with other surface antigen like CD1, CD2, CD3 and CD4.[1-4]

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. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol1983 Aug;131(2):611-6.
2. Knapp W. Leucocyte typing IV : white cell differentiation antigens. Oxford: Oxford University Press; 1989.
3. Marie-Cardine A, Maridonneau-Parini I, Fischer S. Activation and internalization of p56lck upon CD45 triggering of Jurkat cells. Eur J Immunol1994 Jun;24(6):1255-61.
4. Escribano L, Orfao A, Villarrubia J, Diaz-Agustin B, Cervero C, Rios A, et al. Immunophenotypic characterization of human bone marrow mast cells. A flow cytometric study of normal and pathological bone marrow samples. Anal Cell Pathol1998;16(3):151-9.

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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