Anti-Human CD45RO (UCHL1)

	REF	Σ		[A]	
FITC	45ROF-100T	100 test	20 µL/test	0,05 mg/ml	RUO
PE	45ROPE-100T	100 test	20 µL/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: UCHL1;
Isotype: IgG2a;
Tested application: flow cytometry;
Immunogen: The anti-CD45RO monoclonal antibody derives from IL-2 dependent T cell line, CA1;
Species reactivity: Human, Cross-Reactivity: Chimpanzee, Common Marmoset;
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 CD45RO, clone UCHL1 is a monoclonal antibody intended for the identification and enumeration of CD45RO present on approximately 40% of peripheral blood T lymphocytes 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: Ptprc, B220, CD45, CD45R, GP180, L-CA, LY5, T200, UCHL-1;
Gene ID: 5788;
Molecular weight: 180 kDa.

2. ANTIGEN DETAILS

Large description: The CD45RO antigen is present at low density early in the T-lymphocyte maturation cycle. Upon activation by phytohemagglutinin (PHA) or alloantigen, naive T lymphocytes first acquire CD45RO and then lose CD45RA. When these activated T lymphocytes are rechallenged, the cells that exhibit a secondary response are primarily CD45RO +, leading to the concept that CD45RO + cells are a primed population of memory T lymphocytes.

In peripheral blood, the CD45RO antigen is present on approximately 40% of resting peripheral blood T lymphocytes, including the CD4 + and CD8 + subpopulations, as well as on most thymocytes and activated T lymphocytes. It is also expressed on monocytes, macrophages, and granulocytes.[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.

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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 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. Norton AJ, Ramsay AD, Smith SH, Beverley PC, Isaacson PG. Monoclonal antibody (UCHL1) that recognises normal and neoplastic T cells in routinely fixed tissues. J Clin Pathol1986 Apr;39(4):399-405.
2. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PC. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology1986 May;58(1):63-70.
3. Akbar AN, Terry L, Timms A, Beverley PC, Janossy G. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol1988 Apr 1;140(7):2171-8.
4. Ledbetter JA, Tonks NK, Fischer EH, Clark EA. CD45 regulates signal transduction and lymphocyte activation by specific association with receptor molecules on T or B cells. Proc Natl Acad Sci U S A1988 Nov;85(22):8628-32.
5. Streuli M, Morimoto C, Schrieber M, Schlossman SF, Saito H. Characterization of CD45 and CD45R monoclonal antibodies using transfected mouse cell lines that express individual human leukocyte common antigens. J Immunol1988 Dec 1;141(11):3910-4.

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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