Anti-Human CD8 (143-45)

	REF	Σ		[A]	
PURE	8PU1	1 mg	1 mg/ml		
PerCP-Cyanine5.5	8PPC5.5-100T	100 test	5 µL/test	0,2 mg/ml	RUO
APC-C750	8AC750-100T	100 test	5 µL/test	0,2 mg/ml	
FITC	8FI-X-100T	100 test	20 µL/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: 143-44;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-CD8 monoclonal antibody derives from T cells;
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 CD8, clone 143-44, is a monoclonal antibody intended for the identification and enumeration of human T cells suppressor/cytotoxic 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: T8, Leu2;
Gene ID: 925;
Molecular weight: 30/32 kDa.

2. ANTIGEN DETAILS

Large description: The monoclonal antibody is directed against the CD8-antigen (T8-antigen), which is expressed on human T lymphocytes. The monoclonal antibody reacts with 20-30% of human peripheral T lymphocytes. The monoclonal antibody reacts with T lymphocytes with suppressor-cell activity in pokeweed mitogen- stimulated immunoglobulin production, as was shown in separation experiments (i.e., "panning"). The monoclonal antiody does not react with B-cells, monocytes, granulocytes and platelets.[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. Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, Cotsarelis G. Human hair follicle bulge cells are biochemically distinct and possess an epithelial stem cell phenotype. J Investig Dermatol Symp Proc1999 Dec;4(3):296-301.
2. Mason DY, Cordell JL, Gaulard P, Tse AG, Brown MH. Immunohistological detection of human cytotoxic/suppressor T cells using antibodies to a CD8 peptide sequence. J Clin Pathol1992 Dec;45(12):1084-8.
3. Nuckols JD, Shea CR, Horenstein MG, Burchette JL, Prieto VG. Quantitation of intraepidermal T-cell subsets in formalin-fixed, paraffin-embedded tissue helps in the diagnosis of mycosis fungoides. J Cutan Pathol1999 Apr;26(4):169-75.
4. Takahashi K, Nakata M, Tanaka T, Adachi H, Nakauchi H, Yagita H, et al. CD4 and CD8 regulate interleukin 2 responses of T cells. Proc Natl Acad Sci U S A1992 Jun 15;89(12):5557-61.
5. Yamagata K, Tanaka M, Kudo H. A quantitative immunohistochemical evaluation of inflammatory cells at the affected and unaffected sites of inflammatory bowel disease. J Gastroenterol Hepatol1998 Aug;13(8):801-8.

7. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for > test
	Regulatory Status
	Quantity per test
RUO	Research Use Only
[A]	Concentration
	Manufacturer

8. MANUFACTURED BY:

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