

Anti-Human CD25 (TP1/6)

Fluorochrome	Reference	Test
FITC	25F-100T	100 test
PE	25PE-100T	100 test



PRODUCT DESCRIPTION

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.

Description: The anti-CD25 monoclonal antibody derives from T cell blasts activated with PMA and ionomycin.

Clone: TP1/6

Isotype: Mouse IgG2b, kappa

Reactivity: Human

Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.

Purification: Affinity chromatography.

Composition: Mouse anti-human CD25 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN₃).

Fluorochrome	Reagent provided	Concentration (μ g/ml)
FITC (Fluorescein isothiocyanate)	100 ug in 2 ml	50
PE (R-Phycoerythrin)	25 ug in 2 ml	12,5

RECOMMENDED USAGE

Immunostep's CD25, clone TP1/6, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample leucocytes that express CD25 using flow cytometry.

CLINICAL RELEVANCE

The CD25 antigen is also present on malignant cells from patients with human T cell lymphotropic virus-associated lymphoma/leukemia⁽¹⁻³⁾

PRINCIPLES OF THE TEST

The anti-CD25 monoclonal antibody binds to the surface of cells that express the CD25 antigen. To identify these cells, the sample is incubated with the antibody and is analysed by flow cytometry.

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the date indicated.

Once the vial is open, the product is stable for 90 days.

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS

- The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
- Avoid microbial contamination of the reagent.
- Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
- Never mouth pipette.
- In the case of contact with skin, wash in plenty of water.
- The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- FOR *IN VITRO* DIAGNOSTIC USE.
- For professional use only.
- Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)^{4,5}. For optimum results, the sample should be processed during the six hours following the extraction. Samples which cannot be processed within the 48 hours following the extraction should be discarded.

MATERIALS REQUIRED BUT NOT PROVIDED

- Isotype controls:

Fluorochrome	Isotype control	Immunostep Reference
FITC	Mouse IgG2b	ICIGG2BF-100UG
PE	Mouse IgG2b	ICIGG2BPE-50UG

- Centrifuge
- Commonly used 12 x 75-mm flow cytometry assay tubes
- Micropipettes for dispensing volumes from 5 µl to 2 ml
- Blood collection tubes with anticoagulant.
- Phosphate buffered saline (PBS) with 0.09% sodium azide. It is recommendable to add 0.5% BSA
- Vacuum system
- Lysing solution
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

SAMPLE PREPARATION:

1. Add the suggested volume indicated on the antibody vial to a 12x75-mm cytometer tube. It is advisable to prepare an additional tube with the appropriate isotype control (*please see materials required but not provided*).
2. Add 100 µL of sample (up to 10⁶ cells) and mix properly in the vortex.
3. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
4. Add 2 ml of the lysing solution, mix in the vortex and incubate in the dark for 10 minutes or until the sample is lysed.
5. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
6. Resuspend pellet.
7. Add 2 ml of PBS (*please see materials required but not provided*).
8. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
9. Resuspend the pellet in 0.3 ml of PBS.

Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out. Samples should be acquired within the 3 hour after lysis.

FLOW CYTOMETRY ANALYSIS

Collect the fluorescence attributed to monoclonal antibody CD25 and determine the percentage of stained cells.

It is necessary to use an isotype control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD25, so as to evaluate and correct the unspecific binding of leucocytes (*please see materials required but not provided*). Set an analysis region to eliminate fluorescence background noise and to include positively stained cells.

Below is an example diagram of stained cells:

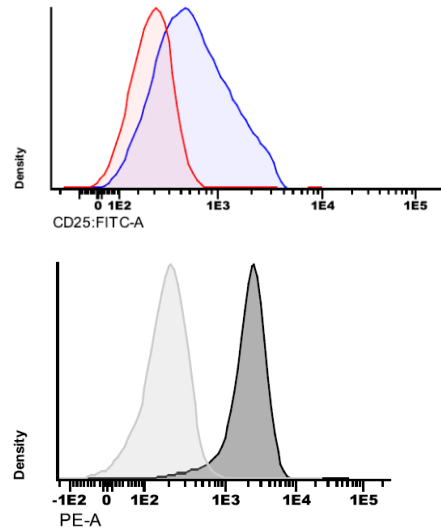


Fig. 1 Average fluorescence intensity of the CD25+ lymphocyte population PMA and PHA activated cells (12 H) in histogram format.

LIMITATIONS OF THE PROCEDURE

1. Incubation of antibody with cells for other than the recommended procedures may result in a reduction or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.
3. Abnormal cells or cell lines may show a higher antigen density than normal cells. In some cases, this could require the use of a greater quantity of monoclonal antibody than is indicated in the procedures for sample preparation.
4. In whole blood samples, red blood cells found in abnormal samples, as well as nucleated red cells (from both normal and abnormal specimens) may be resistant to lysis. Longer periods of red blood cell lysing may be needed in order to avoid the inclusion of unlysed cells in the lymphocyte gated region.
5. Blood samples should not be refrigerated for an extensive period (more than 24 hours), since the number of viable cells will gradually decrease, and this may have an effect on the analysis. In order to obtain the best values, they should be kept at room temperature immediately prior to incubation with the monoclonal antibody.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

REFERENCE VALUES

Abnormal results in the percentage of cells expressing the antigen or in its levels of expression may be due to pathological conditions. It is advisable to know the normal antigen expression patterns in order to ensure a proper interpretation of the result^{B,9}

The values obtained from healthy individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.

CHARACTERISTICS

SPECIFICITY

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD25 monoclonal antibody. Cells contained in the lymphocyte, monocyte, granulocyte, platelets and erythrocytes regions were selected for analysis. Blood samples were processed by a leukocyte method, with a direct immunofluorescence staining for flow cytometric analysis.

To evaluate the reagent’s Specificity (cross-reactivity with other cell populations), 10 blood samples from healthy donors were studied, stained with an adequate isotype control and the MAb to study. The percentage of lymphocytes, monocytes, granulocytes, platelets and erythrocytes stained with the mentioned MAb was evaluated.

The results obtained for CD25 FITC are shown in the following table:

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
% lymphocytes	10	15,08	31,37	22,16	6,8052
% Monocytes	10	11,52	27,76	17,97	6,1295
% Granulocytes	10	7,59	31,42	17,17	8,5597
Valid N (listwise)	10				

SENSIBILITY

Sensitivity of the Immunostep CD25 monoclonal antibodies was determined by staining a blood sample from donor. Dilutions of a peripheral blood sample were made to check the concentration scale of stained cells obtained. The results show an excellent correlation level between the results obtained and expected based on the dilution used.

To determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,997(a)	,995	,994	,37577

a Predictors: (Constant), Expected

REPRODUCIBILITY

Reproducibility for the Immunostep CD25 conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD25+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD25. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes were selected for the

analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from a normal donor expressing a high percentage of CD25+ cells. Mid-range and low range samples were obtained by mixing known CD25- cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples.

Descriptive Statistics						
FITC						
	N		Minimum	Maximum	Mean	Std. Deviation
High	10	10	11,9	13,54	12,4940	0,5063
Medium	10	10	5,48	7,49	6,0937	0,7251
Low	10	10	2,58	3,52	2,9136	0,3233
Valid N (listwise)	10					
PE						
High	10	10	16,04	18,83	17,3880	,84355
Medium	10	10	11,54	13,00	12,0370	,49477
Low	10	10	2,85	3,56	3,1910	,18586
Valid N (listwise)	10					

*Note: Data analyzed with SPSS for Windows 11.0.1

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.

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MANUFACTURED BY



Immunostep S.L

Avda. Universidad de Coimbra, s/n
Cancer Research Center (CIC)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. (+34) 923 294 827
www.immunostep.com