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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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siehe unsere Liefer- und Versandbedingungen

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Product: FITC Anti-Human CD25
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.
Cat. Ref: 25F-100T
Reagent provided: 100 test (20µl /test)
Description: Mouse monoclonal antibody anti-human CD25 FITC is recommended for use in flow cytometry for identification of activated T cells and B cells, monocytes/macrophages, Treg. The antibody is provided in liquid form in buffer containing aqueous buffered solution with protein stabilizer and ≤0.09% sodium azide.
Clone: TP1/6
Isotype: IgG2b, kappa
Immunogen: T cell blasts activated with PMA and ionomycin
Cross-Reactivity: Chimpanzee
Fluorochrome: FITC (Ex-Max: 494 nm/Em-Max: 520 nm). The fluorophore is excited with the blue laser (488 nm) ion argon laser. It is recommended to use a 502 LP dichroic mirror and 530/30 band pass filter detector-equipped flow cytometer

Storage: Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com).

Application: It is recommended for use in flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 20 µl/10^6 cells.

INTENDED PURPOSE.

CD25 FITC is a monoclonal antibody conjugated that may be used to identification of epitope B of the IL-2 alpha receptor in peripheral blood.

TECHNICAL SUMMARY.

Reactivity: Antibody recognizes a type I transmembrane glycoprotein also known as the low affinity IL-2 receptor α chain or Tac. CD25 is a epitope B of the IL-2 alpha receptor of about 55 kDa. Expression of human CD25 (the alpha subunit of the IL-2 receptor complex) is dramatically upregulated on activated T cells, B cells, NK cells and macrophages

Specificity: The CD25 antigen is present on normal T lymphocytes which are activated and functionally mature, monocytes, and myeloid progenitors. It is not present on resting T, B and null cells, except B cells stimulated with anti-IgM antibody.

CLINICAL RELEVANCE

The CD25 antigen is also present on malignant cells from patients with human T cell lymphotropic virus-associated lymphoma/leukemia.

PRINCIPLES OF THE TEST.

Immunostep CD25 FITC monoclonal antibodies bind to the surface of cells that express the CD25 antigen. To identify these cells, peripheral blood leucocytes are incubated with the antibodies and red blood cells are lysed before washing to remove unbound antibodies. An appropriate fixative solution is added to lysed, washed cells before the stained and fixed cells are analysed by flow cytometry with an Helio-Neon laser at 488 nm.

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REAGENTS.

Cluster Designation:	Anti-Human CD25
Clone:	TP1/6
Isotype:	IgG2b
Species:	Mouse
Composition:	IgG2b heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	FITC Excitation wavelength 488 nm Emission wavelength 520 nm
Molar composition:	FITC/protein ±6.0 (3-8)
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests, containing protein stabilizer and ⩽0.09% sodium Azide, 0.02 M sodium phosphate, 0.15 M sodium chloride, pH 7.2
Reagent preparation:	Ready to use.

1. STATEMENTS, SETTINGS AND WARNINGS.

- Reagents contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
- Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.
- Do not pipet by mouth.
- Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.
- The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes.
- Do not use antibodies beyond the stated expiration dates of the products.
- Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing.
- FOR *IN VITRO* DIAGNOSTIC USE
- For professional use only.

2. APPROPIATE STORAGE CONDITIONS.

a. FITC
Keep in dark place at 2-8°C. DO NOT FREEZE.

3. EVIDENCE OF DETERIORATION.

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

- The normal appearance of the FITC conjugated monoclonal antibody is a clear, yellow liquid without aggregates or precipitates.

4. SPECIMEN COLLECTION.

Collect venous blood samples into blood collection tubes using an appropriate anticoagulant (EDTA or heparin). For optimal results the sample should be processed within 6 hours of venepuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of

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venepuncture. ACD or heparin, but not EDTA, may be used if the sample is not processed within 30 hours of venepuncture. Samples that cannot be processed within 48 hours should be discarded.

If venous blood samples are collected into ACD for flow cytometric analysis, a separate venous blood sample should be collected into EDTA if a CBC is required.

Unstained anticoagulated blood should be retained at 20- 25°C prior to sample processing. Blood samples that are haemolysed, clotted or appear to be lipemic, discoloured or to contain interfering substances should be discarded.

Refer to "*Standard Procedures for the Collection of Diagnostic Blood Specimens*" published by the National Committee for Clinical Laboratory Standards (NCCLS) for additional information on the collection of blood specimens.

5. SAMPLE PREPARATION.

Staining Cell Surface Antigens for Flow Cytometry Protocol

1. Add 20 µL of CD25 FITC to a 12 x 75 mm polystyrene test tube. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory
2. Transfer 100 µL of anticoagulated (EDTA) blood (10^6 cells) and mix gently with a vortex mixer.
3. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype.
4. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.
5. Add Lysing Solution according to the manufacturer's directions to each sample and mix gently with a vortex mixer.
6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.
7. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
8. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: **www.immunostep.com**

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents:	Mouse IgG2b: FITC
Leucocyte gating reagent:	Mouse anti-human CD45: PerCP

Serofuge or equivalent centrifuge

12 x 75 mm polypropylene centrifuge tubes

Micropipette capable of dispensing 5 µl, 20 µl, 100 µl, and 500 µl volumes

Blood collection tubes with anticoagulant

Phosphate buffered saline (PBS)

Trypan Blue or propidium iodide, 0.25% (w/v) in PBS for the determination of cell viability

Lysing Solution

Fixing Solution

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Flow cytometer:	Becton Dickinson FACSCaliburTM, Coulter Profile or equivalent 488 nm ion argon laser, 502 LP filter and 530/30 detector-equipped flow cytometer.

7. INTERPRETATION OF RESULTS.

a. *FLOW CYTOMETRY*

Analyze antibody-stained cells on an appropriate flow cytometer analyzer according to the manufacturer instructions. The right angle light scatter or other scatter (SSC) versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the FITC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained T lymphocytes is determined. An appropriate FITC-conjugated isotypic control of the same heavy chain immunoglobulin class and antibody concentration must be used to estimate and correct for non-specific binding to lymphocytes. An analysis region is set to exclude background fluorescence and to include positively stained cells. The following histograms are representative of cells stained and region from a normal donor.

8. QUALITY CONTROL PROCEDURES.

Non-specific fluorescence identified by the FITC conjugated isotypic control is usually less than 2% in normal individuals. Non-specific fluorescence identified by the PE and APC conjugated isotypic controls are usually less than 4% in normal individuals. If the background level exceeds these values, test results may be in error. Increased non-specific fluorescence may be seen in some disease states.

A blood sample from each normal and abnormal donor should be stained with the CD45 Pan-leukocyte and CD14 Pan-monocyte monoclonal antibodies. When used in combination, these reagents assist in identifying the lymphocyte analysis region, and distinguish lymphocytes from monocytes, granulocytes and unlysed or nucleated red cells and cellular debris.

A blood sample from a healthy normal donor should be analyzed as a positive control on a daily basis or as frequently as needed to ensure proper laboratory working conditions. Each laboratory should establish their own normal ranges, since values obtained from normal samples may vary from laboratory to laboratory.

An appropriate isotype control should be used as a negative control with each patient sample to identify non-specific Fc binding to lymphocytes. An analysis region should be set to exclude the non-specific fluorescence identified by the isotypic control, and to include the brighter fluorescence of the lymphocyte population that is identified by the specific antibody.

Refer to the appropriate flow cytometer instrument manuals and other available references for recommended instrument calibration procedures.

9. LIMITATIONS OF THE PROCEDURE.

1. Incubation of antibody with cells for other than the recommended time and temperature may result in capping or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
3. Abnormal cells or cell lines may have a higher antigen density than normal cells. This could, in some cases, require the use of a larger quantity of monoclonal antibody than is indicated in the procedures for Sample Preparation.
4. Blood samples from abnormal donors may not always show abnormal values for the percentage of lymphocytes stained with a given monoclonal antibody. Results obtained by flow cytometric analysis should be considered in combination with results from other diagnostic procedures.
5. When using the whole blood method, red blood cells found in some abnormal donors, as well as nucleated red cells found in normal and abnormal donors may be resistant to lysis by lysing solutions. Longer red cell lysis periods may be needed to avoid the inclusion of unlysed red cells in the lymphocyte gated region.

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6. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24-30 hours) prior to incubating with monoclonal antibodies.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the laser, as well as proper gate settings.
8. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.
9. Al results need to be interpreted in the context of clinical features, complete immunophenotype and cell morphology, taking due account of samples containing a mixture of normal and neoplastic cells.

10. REFERENCE VALUES.

The cellular elements of human Bone Marrow include lymphocytes, monocytes, granulocytes, red blood cells and platelets.

Nucleated cells Percentage in the Bone Marrow

Cell type	Percentage
Progranulocytes	56,7
Neutrophils	53,6
Myeloblasts	0,9
Promyeloblasts	3,3
Promyelocytes	12,7
Metamyelocytes	15,9
Eosinophils	3,1
Basophils	<0,1
Proerythrocyte	25,6
Proerythrblasts	0,6
Basophil Erythroblast	1,4
Polycromatic Erythroblast	21,6
Ortocromatic Erythroblast	2
Megakaryocytes	<0,2
Lymphocytes	16,2
Plasma cells	2,3
Reticular cells	0,4

Normal human peripheral blood lymphocytes 20-47% (n=150% confidence interval)

Nucleated cells Percentage in Peripheral Blood of a Normal Patient

Cell type	Percentage	Number of event.
Red Blood Count		3,8 - 5,6 $X10^6$/µL
Platelets		150 - 450 $X10^3$/µL
White Blood Count (WBC)		4.3 - 10.0 $X10^3$/µL
Neutrophils	57 – 67 %	1,5 - 7.0 $X10^3$/µL
Lymphocytes*	25 – 33 %	1.0 - 4.8 $X10^3$/µL
T cell	56 – 82 % of lymphocytes	
T cell CD4+	60 % of T cells	
T cell CD8+	40 % of T cells	
Cell NK+	6 – 33 of lymphocytes	
B cell	7.7 – 22 of lymphocytes	
Monocytes	3 – 7 %	0.28 - 0.8 $X10^3$/µL
Eosinophils	1 – 3 %	0.05 – 0,25 $X10^3$/µL
Basophils	0 – 0,075 %	0,015 – 0,05 $X10^3$/µL
Reticulocyte	0,5 – 1,5 % of total Red Blood Cell	

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Expected values for pediatrics and adolescents have not been established.

The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

11. PERFORMANCE CHARACTERISTICS.

a. *SPECIFICITY*

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD25 FITC 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.

The results obtained are shown in the following table:

Descriptive Statistics

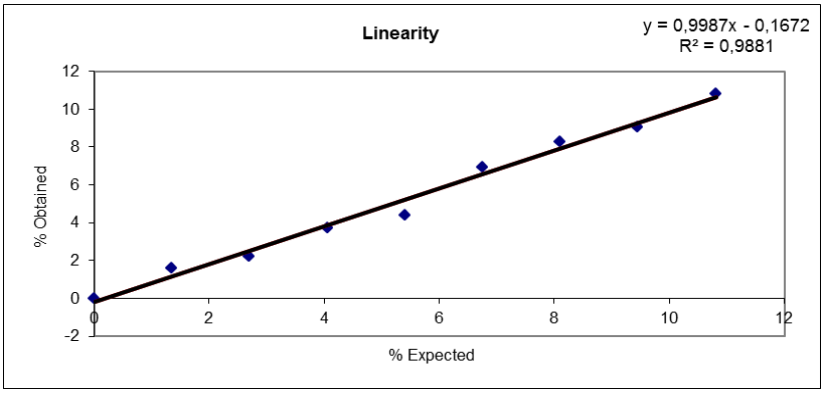
	N	Minimum	Maximum	Mean	Std. Deviation
% lymphocytes	10	15,08	31,37	22,16	6,8052
% Monocytes	10	11,52	27,76	17,97	6,1295
% Granulocytes	10	7,59	31,42	17,17	8,5597
Valid N (listwise)	10				

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.

b. *SENSIBILITY or LINEARITY*

Sensitivity of the Immunostep CD25 FITC 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



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c. REPRODUCIBILITY

Reproducibility for the Immunostep CD25 FITC-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each sample in each of three leukocyte ranges: high, medium and low. Three samples of each range were used. Thus, a total of 30 determinations were performed for each type of percentage. Thereby reproducibility was demonstrated throughout the entire measuring range.

The 30 determinations for each range were performed by the staining, processing and analysis of 3 separate samples. Lymphocytes CD25+ were selected for the analysis of percentage cells stained in each measure.

To perform this study, anti-coagulated blood was obtained from normal donors expressing a different percentage of leukocytes.

Descriptive Statistics

Range	N		Minimum	Maximum	Mean	Std. Deviation
	Porcentage	Valid N (listwise)	Porcentage	Porcentage	Porcentage	Porcentage
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

12. BIBLIOGRAPHY.

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3. Chung IY, Dong HF, Zhang X, Hassanein NM, Howard OM, Oppenheim JJ, Chen X. Effects of IL-7 and dexamethasone: Induction of CD25, the high affinity IL-2 receptor, on human CD4(+) cells. Cell Immunol. 2004 Nov-Dec;232(1-2):57-63.