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- Mindermengenzuschlag
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Product: PE Anti-Human CD25

Cat. Ref: 25PE-100T

Reagent provided: 100 test (20µl / test)

Description: Monoclonal Mouse Anti-Human CD25 PE, is recommended for use in flow cytometry for identification of human receptor for Interleukin-2 (IL-2R) expressing the 55,000 M.W. surface antigen. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide.

Clone: TP1/6

Isotype: IgG2b

Fluorochrome: R-Phycoerythrin (R-PE). PE (Ex.: 496, 564 nm/Em-Max: 578 nm). Recommended 488 nm ion argon laser, 556 LP filter and 585/42 or 575/26 detector-equipped flow cytometer.



INTENDED PURPOSE.

CD25 PE is a monoclonal antibody conjugated that may be used to identification for identification of human receptor for Interleukin-2 (IL-2R) expressing the 55,000 M.W. surface antigen.

TECHNICAL SUMMARY.

Reactivity: Antibody recognizes epitope B of the IL-2 alpha receptor of about 55 kd . 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. 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 PE 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.

REAGENTS.

Cluster Designation:	CD25
Clone:	TP1/6
Isotype:	IgG2b
Species:	Mouse
Composition:	IgG2b heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	R- Phycoerythrin (PE) Excitation wavelength 488 nm Emission wavelength 575 nm
Molar composition:	PE/protein ±1.0
Reagents contents:	2 ml vial containing monoclonal antibody for 100 test, and it is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide 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. APPROPRIATE STORAGE CONDITIONS.

- a. R-Phycoerythrin (RPE)
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 PE conjugated monoclonal antibody is a clear, pink-red liquid.

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 venipuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of venipuncture. ACD or heparin, but not EDTA, may be used if the sample is not processed within 30 hours of venipuncture. 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- 25oC prior to sample processing. Blood samples that are hemolyzed, 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.

LYMPHOCYTES ACTIVATED

1. From a collect blood into an appropriate anticoagulan mixed with EDTA (until the process moment, keep in cold). Determine cell viability using Trypan Blue or propidium iodide. If the cell viability is not at least 85%, the blood sample should be discarded.
2. Pipette 100µl of well mixed blood into 12 x 75 mm polypropylene centrifuge tubes marked unknown and control.

3. Add 20µl of Immunostep CD25PE-conjugated monoclonal antibody and 180µl of phosphate buffered saline (PBS) to tubes marked unknown. In other control tube add 10µl of corresponding Immunostep IgG2b PE-conjugated isotypic control reagent. Mix gently.
4. Incubate all tubes for 15 minutes at room temperature (22 ±3°C) in the dark.
5. Add lysing solution to all tubes according to the manufacturer's directions.
6. Centrifuge all tubes at 400 x g for 3 minutes at room temperature.
7. Add fixing solution to all tubes according to the manufacturer protocol. Retain cells in fixing solution for not less than 30 minutes at room temperature (22 ±3°C) in the dark.
8. Wash the cells in all tubes twice with 4mL of PBS. Centrifuge at 400 x g for 3 minutes after each wash procedure.
9. Resuspend the cells from the final wash in 1 ml of PBS and store tubes at 2-8°C in the dark until flow cytometric analysis is performed. It is recommended that analysis be performed within 24-48 hours of staining and fixation.
10. Analyze on a flow cytometer according to the manufacturer instructions. For alternate methods of whole blood lysis, refer to the manufacturer recommended procedure.

LYMPHOCYTES NO ACTIVATED

Samples: 1ml of peripheral blood (PB), obtained in a tube containing an anticoagulant that will not chelate calcium (usually PB samples in sodium heparin are used)

Two tubes are labelled for each test: NEGATIVE CONTROL and ACTIVATED

Procedure

A. ACTIVATION:

The following are added to the **NEGATIVE CONTROL** tube

1. 500 mL of culture medium (RPMI 1640) supplemented with 2 mM L-Glutamine.
2. 10 mL of BREFELDIN A is added per mL of culture with PB to block the secretion of proteins to the extracellular space.
3. 500 mL of heparinized blood is added.

The following are added to the **ACTIVATED** tube

1. 500 mL of culture medium (RPMI 1640) supplemented with 2 mM L-glutamine.
2. Cytokine secretion stimulants:
 - a. Lymphocyte activation:
 - i. 20 ng per mL of Phorbol Myristate Acetate (PMA)
 - ii. 1 mg per mL of Ionomycine.
 - b. Activation of dendritic cells and monocytes:
 - i. 100 ng per mL of lipopolysaccharide (LPS)
 - ii. 10 ng per mL of recombinant human interferon-gamma (IFN-g).
3. BREFELDIN A: This is added at a total concentration of 10 mg per mL of culture to block the secretion of proteins to the extracellular space.
4. 500 mL of heparinized blood.

B. INCUBATION

The tubes are incubated at 37°C in 5% CO₂ and 95% humidity in a sterile atmosphere. The incubation time is 4 hours for lymphocytes and 6 hours for monocytes and dendritic cells.

C. MEMBRANE LABELLING

For membrane labelling, which will allow the identification of the different cell subsets to be studied, the direct immunofluorescence technique is used.

1. 200 mL of membrane is added to previously labelled tubes.
2. The recommended amount of McAb is added for the identification of the different cell types to be studied.
3. The samples are shaken with a vortex mixer and are incubated for 5 min in the darkness at room temperature.

D. WASHING

Two mL of PBS is added to each tube and the mixture is centrifuged at 2000 rpm for 5 min at room temperature. The supernatant is removed with a Pasteur pipette or a vacuum pump.

E. ACQUISITION.

The samples are acquired on a flow cytometer in a maximum time of one hour. In the case of dendritic cells, the protocol of the analysis of different cells in general is followed; in a first step, some 30,000 cells corresponding to the total cellularity are acquired and in a second step those cells included in the HLA-DR fraction are acquired, up to a total of 500,000.

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

ISOTYPE CONTROL REAGENTS:

Leucocyte gating reagent:

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

Flow cytometer:

MOUSE IGG2B: PE

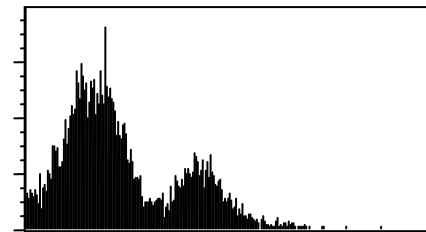
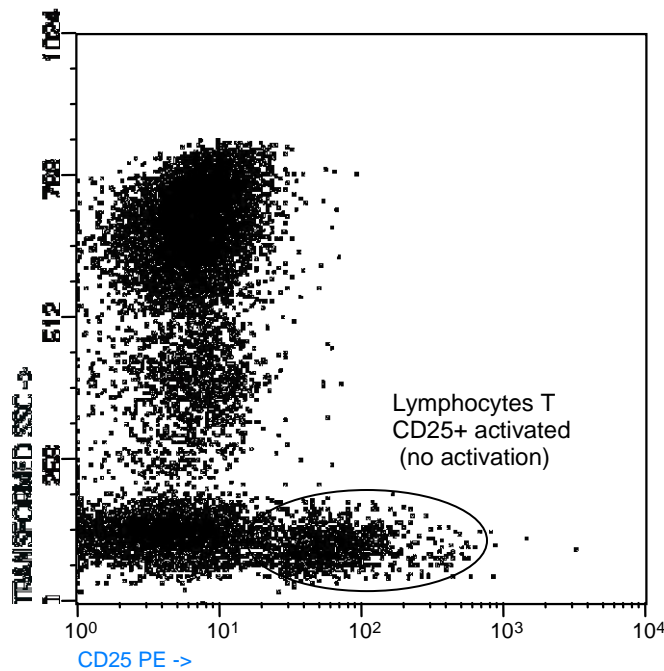
Mouse anti-human CD45: FITC/CD14: PE

Becton Dickinson FACScan™, Coulter Profile
or equivalent.

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 PE- conjugated monoclonal antibody is collected, and the percentage of antibody-stained T lymphocytes is determined. An appropriate PE- 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.



CD25 PE

The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on leucocytes. Human peripheral blood lymphocytes were stained with CD25 PE. Cells were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer, using Cell Quest acquisition software and PAINT-A-GATE. PRO, analysis software.

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-lymphocyte 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.
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. AI 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
Proerythblasts	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 ⁹ /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	

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.

All data were obtained from a normal peripheral blood without activated lymphocytes.

a. SPECIFICITY

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD25PE monoclonal antibody. Cells contained in the lymphocyte, monocyte and granulocyte 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 and granulocytes stained with the mentioned MAb was evaluated. The results obtained are shown in the following table:

Case Summaries

	Lymphocytes	Monocytes	Granulocytes
1	54,77	33,57	24,59
2	53,65	31,11	17,50
3	47,34	30	28,59
4	61,85	23,78	30,26
5	61,75	32,91	30,41
6	66,95	35,37	31,08
7	58,96	28,14	38,57
8	50,26	34,90	32,07
9	49,14	35,28	33,39
10	62,59	34,08	38,82
Total N	10	10	10

Statics

		Lymphocytes	Monocytes	Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		56,7260	31,9140	30,5280
Median		56,8650	33,2400	30,7450
Mode		47,34 (a)	23,78 (a)	17,50 (a)
Std. Deviation		6,63446	3,73171	6,26396
Variance		44,01607	13,92565	39,23720
Range		19,61	11,59	21,32

(a) Multiple modes exist. The smallest value is shown

b. 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

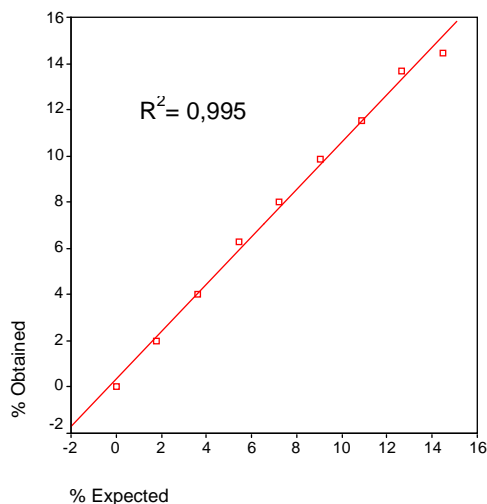
Case Summaries

Sample	Dilution	Expected	Obtained
400µl A + 0µl B	100,00	14,48	14,48
350µl A + 50µl B	87,50	12,67	13,69
300µl A + 100µl B	75,00	10,86	11,52
250µl A + 150µl B	62,50	9,05	9,86
200µl A + 200µl B	50,00	7,24	7,99
150µl A + 250µl B	37,50	5,43	6,30
100µl A + 300µl B	25,00	3,62	4,02
50µl A + 350µl B	12,50	1,81	2,00
0µl A + 400µl B	,00	,00	,00
9	9	9	9

Model Summary

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

a Predictors: (Constant), Expected



c. REPRODUCIBILITY

Reproducibility for the Immunostep CD25: PE-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.

Case Summaries

Sample	High	Medium	Low
SAMPLE 1	16,04	11,56	3,22
SAMPLE 2	17,25	11,78	3,56
SAMPLE 3	17,11	11,66	3,2
SAMPLE 4	18,66	12,19	2,99
SAMPLE 5	18,83	13	3,15
SAMPLE 6	17,16	12,41	3,27
SAMPLE 7	17,16	12,27	3,28
SAMPLE 8	16,91	11,55	2,85
SAMPLE 9	17,86	12,41	3,22
SAMPLE 10	16,9	11,54	3,17
10	10	10	10

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
High	10	16,04	18,83	17,3880	,84355
Medium	10	11,54	13,00	12,0370	,49477
Low	10	2,85	3,56	3,1910	,18586
Valid N (listwise)	10				

**Note: Data analyzed with SPSS for Windows 11.0.1*

12. BIBLIOGRAPHY.

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