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Product: APC Anti-Human CD45

Cat. Ref: 45A1-100T

Reagent provided: 100 test (20µl / test)

Description: Monoclonal Mouse Anti-Human CD45 APC is recommended for use in flow cytometry for identification and analysis of CD45⁺ cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide

Clone: D3/9

Isotype: IgG1

HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code 825.

Fluorochrome: *Allophycocyanin (APC). APC (Ex-Max: 650 nm/Em-Max: 660). Recommended a dye laser (600 nm) or a helium-neon (HeNe) laser (633 nm) and 660/20 detector-equipped flow cytometer.*



INTENDED PURPOSE.

CD45 APC is a monoclonal antibody conjugated that may be used to identification of lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD45-antigen, defined T200 or Leucocyte Common Antigen. The antibody reacts with all cells of the haemopoietic lineage, not with cells of other lineages.

Specificity: The 180, 195, 205, 220, kD MW components of the leucocyte common antigen complex to be found on lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells. No reactivity has been observed with primary or metastatic carcinoma cells. Plasma cells or myeloma cells may have weak expression or be negative for this antigen.

CLINICAL RELEVANCE

CD45 is a critical requirement for T and B cell antigen receptor-mediated activation and possible requirement for receptor-mediated activation in other leukocytes.

This reagent can be used in the characterization studies for immunophenotyping of leucocytes, which are widely applied in the characterization and follow-up of immunodeficiencies, autoimmune diseases, leukemias, etc

CD45 antibody has been used in immunohistochemistry to study the effects of the natural plasma constituent recovered from type 2 diabetic patients (dm-LDL) on endothelial cells and to study the expression, localization, and functional activity of TLIA in inflammatory bowel disease.

Anti-CD45 antibody can be used for study the expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis.

Detection of distinct isoforms can distinguish between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.

Combination of CD45 with CD14 antibodies in the analysis of blood or bone marrow samples by flow cytometry shows variable expression of these antigens on different cell populations studies on the function of individual CD45 with potent immunosuppressive activity, suggesting that CD45 may be a useful target for drug design

PRINCIPLES OF THE TEST.

Immunostep CD45 APC monoclonal antibodies bind to the surface of cells that express the CD45 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 633 nm.

REAGENTS.

Cluster Designation:	CD45
WHO Classification:	Leukocyte Workshop IV.
Clone:	D3/9
Isotype:	IgG1
Species:	Mouse
Composition:	IgG1 heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	R-Allophycocyanin (APC) Excitation wavelength 633 nm Emission wavelength 664 nm
Molar composition:	APC/protein ± 1.0
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests and it is provided in aqueous buffered solution containing protein stabilizer, and $\leq 0.09\%$ 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.

Allophycocyanin (APC)
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 APC conjugated monoclonal antibody is a clear deep blue 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.

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 CD45 APC-conjugated monoclonal antibody and 180µl of phosphate buffered saline (PBS) to tubes marked unknown. In other control tube add 10µl of corresponding Immunostep IgG2a APC-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.

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents:

Mouse IgG1: APC

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

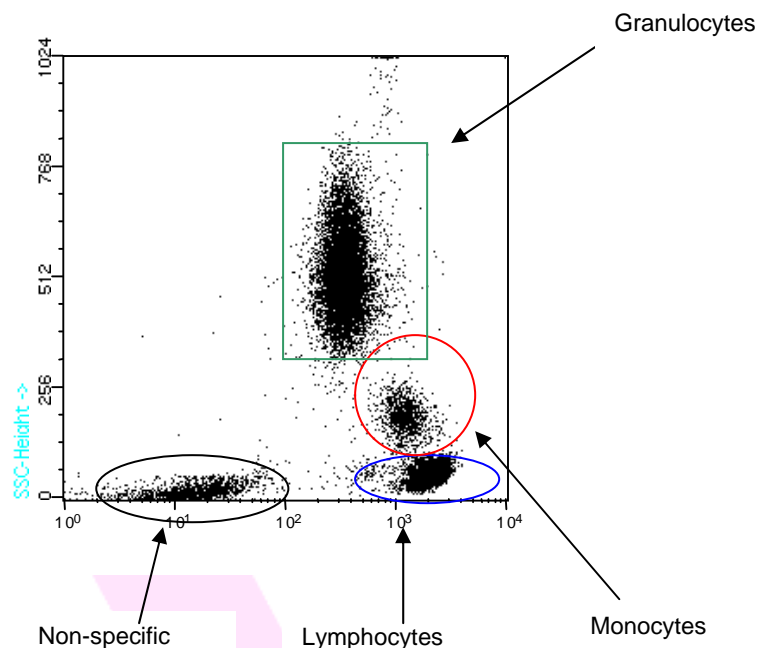
Flow cytometer:

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 APC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained lymphocytes, monocytes, granulocytes, thymocytes and malignant T and B cells is determined. An appropriate APC- 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.



The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample. Human peripheral blood lymphocytes were stained with CD45 APC. 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, monocytes and granulocytes. 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, monocytes and granulocytes 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
Polychromatic 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	

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.

II. PERFORMANCE CHARACTERISTICS.

a. SPECIFICITY

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD45APC 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

	Sample	Lymphocytes	Monocytes	Granulocytes
1	1	94,73	99,90	99,95

2	2	93,55	94,19	99,97
3	3	91,96	99,18	99,98
4	4	94,35	97,20	99,95
5	5	90,58	97,92	99,77
6	6	96,59	83,36	99,97
7	7	95,42	90,80	99,82
8	8	88,95	82,15	99,91
9	9	95,56	82,42	99,90
10	10	95,60	98,65	99,97
Statics	Total N	10	10	10

		Lymphocytes	Monocytes	Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		93,7290	92,5770	99,9190
Median		94,5400	95,6950	99,9500
Mode		88,95(a)	82,15(a)	99,97
Std. Deviation		2,47660	7,35320	,07141
Variance		6,134	54,070	,005
Range		7,64	17,75	,21

a Multiple modes exist. The smallest value is shown

b. SENSIBILITY

Sensitivity of the Immunostep CD45 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

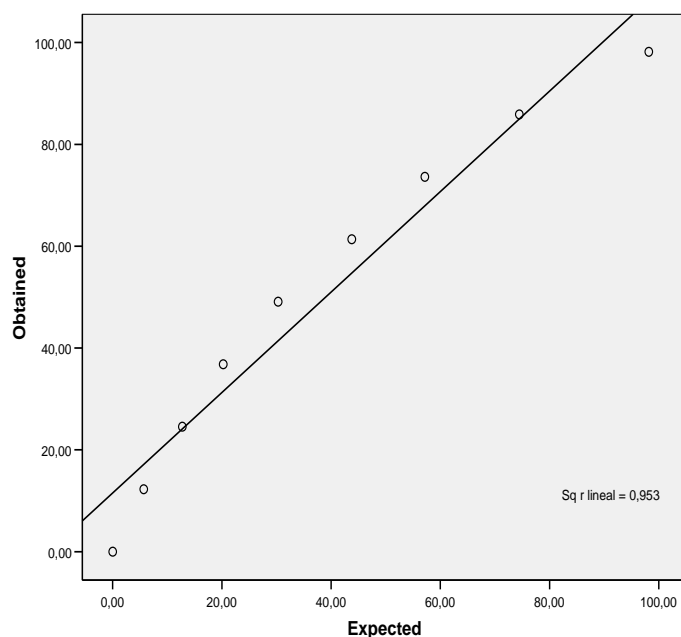
	Sample	Dilution	Obtained	Expected
1	400µl A + 0µl B	100,00	98,18	98,18
2	350µl A + 50µl B	87,50	85,90	74,47
3	300µl A + 100µl B	75,00	73,63	57,16
4	250µl A + 150µl B	62,50	61,36	43,78
5	200µl A + 200µl B	50,00	49,09	30,30

6	150µl A + 250µl B	37,50	36,81	20,25
7	100µl A + 300µl B	25,00	24,54	12,75
8	50µl A + 350µl B	12,50	12,27	5,68
9	0µl A + 400µl B	,00	,00	,00
Total	N	9	9	9

Model Summary

Model	R	R Square	R Square	Adjusted R
1	,976(a)	,953	,946	,79558

a Predictors: (Constant), Expected



c. REPRODUCIBILITY

Reproducibility for the Immunostep CD45 APC-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD45+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD45. 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 CD45+ cells. Mid-range and low range samples were obtained by mixing known CD45- 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
1	1	29,97	18,68	5,81
2	2	29,43	19,74	6,44
3	3	29,57	19,26	5,52
4	4	29,44	18,44	5,55
5	5	29,70	18,55	5,57
6	6	29,21	19,55	5,68
7	7	29,99	19,30	5,14
8	8	31,01	19,55	6,07
9	9	29,24	18,98	5,61
10	10	30,70	19,66	6,37
Total	N	10	10	10

Descriptive Statistics

	High	Medium	Low
Mean	29,8260	19,1710	5,7760
Mode	29,21(a)	19,55	5,14(a)
Std.Deviation	,60768	,47985	,40574
Variance	,369	,230	,165
Range	1,80	1,30	1,30
Mínimum	29,21	18,44	5,14
Maximum	31,01	19,74	6,44

12. BIBLIOGRAPHY.

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