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- Diagnostik & molekulare Diagnostik
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- Mindermengenzuschlag
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Product: APC Anti-human CD4
Cat. Ref: 4A-100T
Reagent provided: 100 test (20µl / test)
Description: Monoclonal Mouse Anti-Human CD4 APC is recommended for use in flow cytometry for identification of Helper/Inducer T cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide.
Clone: HP2/6
Isotype: IgG2a
HLDA: 4th International Workshops on Human Leucocyte Differentiation, WS Code 116
Fluorochrome: Allophycocyanin (APC), Europa Bioproducts, Ely, Cambridge.

INTENDED PURPOSE.

CD4 APC is a monoclonal antibody conjugated that may be used to identification of Helper/Inducer T cells in peripheral blood.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD4-antigen (T4-antigen),which is expressed on human peripheral T lymphocytes and 80% of thymocytes. The monoclonal antibody reacts on a low level with human monocytes and macrophages. The monoclonal antibody does not react with B-cells ,granulocytes and thrombocytes.The monoclonal antibody is directed against the CD3- antigen (T3-antigen), which is expressed on human T lymphocytes. The monoclonal antibody reacts with 80-90% human peripheral T lymphocytes and medullary thymocytes. The monoclonal antibody does not react with B-cells, monocytes, granulocytes and platelets. The monoclonal antibody is mitogenic for resting T lymphocytes and it blocks the cytolytic activity of CTL clones.

Specificity: 60Kd MW lymphocyte surface antigen identified by monoclonal antibodies belonging to the CD4 cluster and present on 54% of peripheral blood T lymphocytes, 50% of thymocytes and some malignant cells of T cell origin. Normal B lymphocytes, monocytes or granulocytes do not express surface CD4 antigen although cytoplasmic expression has been observed in monocytes/macrophages. The CD4 positive T lymphocyte subpopulation has been characterised functionally as comprising helper cells active in amplification of immune responses.

CLINICAL RELEVANCE

The Immunostep CD4 monoclonal antibody may also be used, in combination with other indicators, for the diagnosis or prognosis of some immunodeficiency diseases, including Thymic differentiation and immune response.

PRINCIPLES OF THE TEST.

Immunostep CD4 APC monoclonal antibodies bind to the surface of cells that express the CD4 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 a Helio-Neon laser at 633 nm.

REAGENTS.

Cluster Designation:	CD4
WHO Classification:	Leukocyte Workshop IV
Clone:	HP2/6
Isotype:	IgG2a
Species:	Mouse
Composition:	IgG2a heavy chain Kappa light chain

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Source:	Hybridome Cells
Method of Purification:	Column chromatography
Fluorochrome:	Allophycocianin (APC) Excitation wavelength 633 nm Emission wavelength 664 nm
Molar composition:	APC/protein ±1.0
Reagents contents:	2ml vial containing monoclonal antibody for 100 tests, 1% BSA, 0.02 M sodium phosphate, 0.15 M sodium chloride, 0.1% sodium azide, 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.

Allophycocianin (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- 25°C 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.

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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 CD4 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 IgG2a: APC
Leucocyte gating reagent:	Mouse anti-human CD45: FITC/CD14 PE
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 FACSCaliburTM, Coulter Profile or equivalent 488 nm ion argon laser-equipped and appropriate computer hardware and software

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

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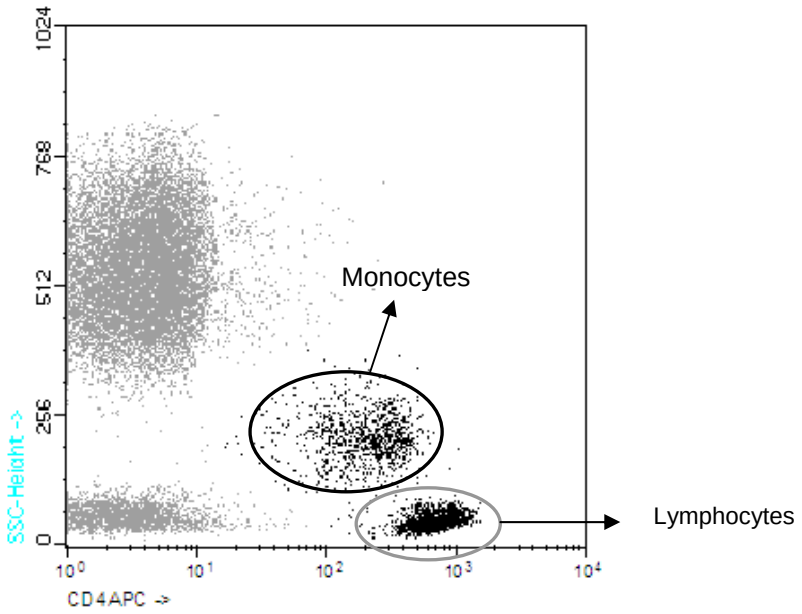
scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the lymphocyte cluster (lymphocyte bitmap). The fluorescence attributable to the APC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained T lymphocytes is determined. An appropriate APC-conjugated 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 gated on the lymphocyte region from a normal donor.



The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on leucocytes. 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 APC conjugated isotypic control is usually less than 2% 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.

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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
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

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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.

a. *SPECIFICITY*

Blood samples were obtained from healthy normal donors of Caucasian were stained with Immunostep CD4 APC 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	40,68	85,74	1,36
2	40,22	72,09	2,12
3	65,8	76,07	1,87
4	42,94	82,93	2,34
5	55,88	69,73	1,78
6	54,95	91,30	2,63
7	34,14	63,88	1,08
8	60,01	71,47	2,86
9	79,07	84,15	2,48
10	88,71	97,33	1,42
Total 10	10	10	10

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Statistics

		Lymphocytes	Monocytes	Granulocytes
N	Valid	10	10	10
	Missing	0	0	0
Mean		56,2400	79,4690	1,9940
Median		55,4150	79,5000	1,9950
Mode		34,14 (a)	63,88 (a)	1,08 (a)
Std. Desviation		17,78565	10,54122	,59201
Variance		316,32933	111,11728	,35047
Range		54,57	33,45	1,78

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

b. *SENSIBILITY*

Sensitivity of the Immunostep CD4 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	15,71	15,71
350µl A + 50µl B	87,50	13,75	14,59
300µl A + 100µl B	75,00	11,78	12,53
250µl A + 150µl B	62,50	9,82	11,24
200µl A + 200µl B	50,00	7,85	8,17
150µl A + 250µl B	37,50	5,89	5,5
100µl A + 300µl B	25,00	3,92	3,57
50µl A+ 350µl B	12,50	1,96	2,66
0µl A + 400µl B	,00	,00	,00
TOTAL	9	9	9

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,995 (a)	,990	,988	,58669

(a) Predictors: (Constant), Expected

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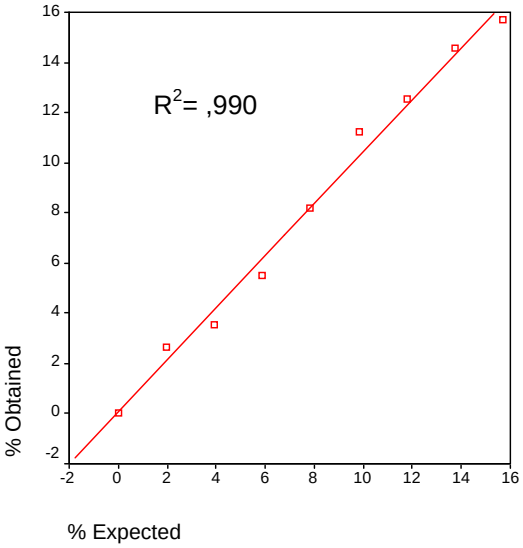

c. *REPRODUCIBILITY*

Reproducibility for the Immunostep CD4: APC-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD4+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD4. 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 CD4+ cells. Mid-range and low range samples were obtained by mixing known CD4- 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	15,34	3,23	1,28
SAMPLE 2	16,06	3,16	1,25
SAMPLE 3	15,34	3,13	1,29
SAMPLE 4	15,51	2,99	1,34
SAMPLE 5	15,5	3,11	1,4
SAMPLE 6	15,69	2,94	1,25
SAMPLE 7	15,53	3,17	1,35

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SAMPLE 8	15,23	3,28	1,44
SAMPLE 9	15,17	3,29	1,35
SAMPLE 10	15,39	3,19	1
	10	10	10

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
High	10	15,17	16,06	15,4760	,25605
Medium	10	2,94	3,29	3,1490	,11387
Low	10	1,00	1,44	1,2950	,12085
Valid N (listwise)	10				

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

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