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Product: PE Anti-Human CD13
Other names: Aminopeptidase N, AP-N, hAPN, Alanyl aminopeptidase, Aminopeptidase M, Microsomal aminopeptidase, Myeloid plasma membrane glycoprotein CD13, gp150
Cat. Ref: 13PE2-100T
Reagent provided: 100 test (20µl / test)
Description: Monoclonal Mouse Anti-Human CD13 for identification of monocytes, neutrophils, eosinophils, and basophils. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide
Clone: WM15
Immunogen: Human AML cells
HLDA: 5th International Workshops on Human Leucocyte Differentiation, WS Code MA191.
Isotype: IgG1, kappa
Fluorochrome: R-Phycoerythrin, R-PE (Ex.: 496, 564 nm/Em-Max: 578 nm). The fluorophore is excited with the blue laser (488 nm), yellow/green (532-561 nm) laser. It is recommended to use a 556 LP filter and 585/42 or 575/26 detector-equipped flow cytometer.

INTENDED PURPOSE.

CD13 PE is a monoclonal antibody that may be used to identification of Aminopeptidase N antigen, expressed by cells of myeloid origin.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD13-antigen, which is expressed on human monocytes, granulocytes and their precursors and a small population of large granular lymphocytes. The monoclonal antibody reacts with monocytes, granulocytes and with a large number of acute myeloid leukaemia's (the positivity of this marker relates to worse clinic prognosis).

Specificity: The CD13 antigen is an aminopeptidase N, 150 to 170 kDa sigle chain integral membrane protein. Present on the surface of early committed progenitors of granulocytes and monocytes (CFU-GM) and by all cells of these lineages as they mature. Expressed on endothelial cells, epithelial cells from renal proximal tubules and intestinal brush border, bone marrow stromal cells, osteoclasts, and cells lining bile duct canaliculi. Expressed on a small proportion of large granular lymphocytes (Soderberg et al 1993) but not other lymphocytes.

CLINICAL RELEVANCE

This antibody is used as a marker for acute myeloid leukemia[1] and plays a role in tumor invasion. In case of human coronavirus 229E (HCoV-229E) infection, serves as receptor for HCoV-229E spike glycoprotein. Mediates as well human cytomegalovirus (HCMV) infection

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.

Precautions:

1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

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PRINCIPLES OF THE TEST.

Immunostep CD13 PE monoclonal antibodies bind to the surface of cells that express the Aminopeptidase N. To identify these cells, peripheral blood leucocytes are incubated with the antibodies. Analysed by flow cytometry with an Helio-Neon laser at 488 nm.

REAGENTS.

Cluster Designation:	Anti-Human CD13
Clone:	WM15
Isotype:	IgG1, kappa
Composition:	IgG1 heavy chain Kappa light chain
Species:	Mouse
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	R-Phycoerythrin Excitation wavelength 488, 532 or 561 nm Emission wavelength 578 nm
Molar composition:	R-PE/protein 1 ± 0,5
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests, 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. R-Phycoerythrin
Keep in dark place at 2-8° C. DO NOT FREEZE. Avoid overexposure to light at room temperature.

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

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- The normal appearance of the R-PE conjugated monoclonal antibody is a clear, pink 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 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 CD13 PE 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 or the study sample (10^6 cells) and mix gently with a vortex mixer.
3. The recommended negative control is a non-reactive PE-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 IgG1: PE
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)

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

Lysing Solution

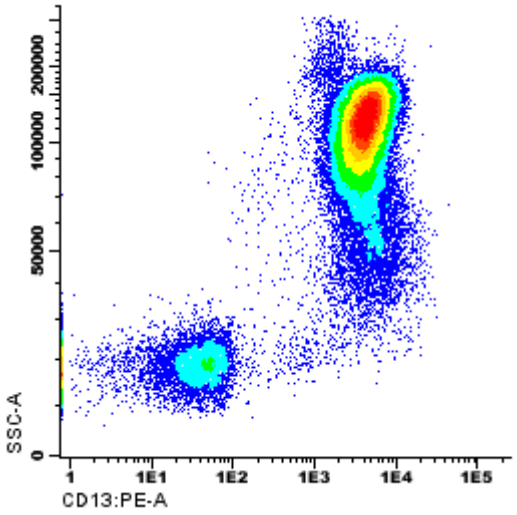
Fixing Solution

Flow cytometer: The equipment must be equipped with a 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 analyser 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 leukocytes cell cluster. A gate is drawn for the Leukocytes CD45+. The fluorescence attributable to the PE-conjugated monoclonal antibody is collected, and the percentage of antibody-stained 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. 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 gated on leucocytes. Human peripheral blood lymphocytes were stained with CD13 PE and CD45 PerCP. Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva 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-leukocyte and CD14 Pan-monocyte monoclonal antibodies. When used in combination, these reagents assist in identifying the cells 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.

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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. All 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
Proerythroblasts	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)

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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.

11. PERFORMANCE CHARACTERISTICS.

a. *SPECIFICITY*

Anti CD13 clone MW15, was included in the fifth International Workshops on Human Leucocyte Differentiation Antigens, WS Code MA191.

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.

Blood samples obtained from healthy normal donors of Caucasian were stained with Immunostep CD13 PE monoclonal antibody. Non-specific fluorescence identified by the PE conjugated isotypic control IgG1 was analysed. Cells contained in the T and B lymphocytes, monocytes, platelets and erythrocytes regions were selected for analysis. Blood samples were processed by a Staining Cell Surface Antigens for Flow Cytometry Protocol.

The results obtained are shown in the following table:

Statistics

		% Isotype control	% T Lymphocytes	% B Lymphocytes	% Platelets	% Erythrocytes
N	Valid	10	10	10	10	10
	Missing	0	0	0	0	0
Mean		,0900	,0110	,0440	,0590	,0890
Std. Deviation		,05270	,00738	,02366	,03035	,08621
Minimum		,02	,00	,02	,01	,01
Maximum		,16	,02	,10	,09	,30

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b. SENSIBILITY or LINEARITY

Sensitivity of the Immunostep CD13 PE monoclonal antibody was determined by staining as positive the U937 cell line and as negative the Jurkat cell line. Cells were mixed in different proportions with a constant final number of 1 x 10^6 cells to achieve different cell ratios from 0% positive cells to 100%.

Thereafter cells were incubated with the antibody according to the recommended amount for 15 minutes. Finally the cells were washed according to standard protocol. A linear regression between the expected values and the observed values was calculated.

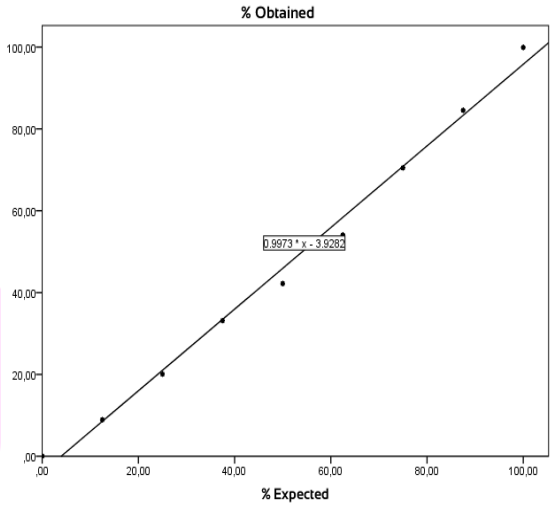
To determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use.

Model Summary[b]

R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
,996[a]	,993	,992	3,12516	y = 0,997x – 3,928

a. Predictors: (Constant), % Expected

b. Dependent Variable: % Obtained



The results show an excellent correlation between the results obtained and expected based on the dilution used. CD13 PE sensibility was demonstrated from 1 x 10^5 to 1 x 10^6 cells in 1 x 10^6 total cells.

c. REPRODUCIBILITY

Reproducibility for the Immunostep CD13 PE-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of three leukocyte ranges: high, medium and low. One sample of each range was used. Thus, a total of 10 determinations were performed for each type of range. Thereby reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 3 separate samples. Cells CD13+ 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.

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Statistics

			Percentage		
			High	Medium	Low
Valor	N	Valid	10	10	10
		Missing	0	0	0
	Mean		75,2965	69,6580	94,3780
	Std. Deviation		,43450	,39919	,35039
	Minimum		74,57	69,06	93,91
	Maximum		75,81	70,18	94,94

The results demonstrate high reproducibility of measurements independent of the values of total leukocytes.

d. ACCURACY or REPEATABILITY

To determine the repeatability of staining with this product, 10 different samples were stained with two different lots of this reagent. For each sample two different values were obtained: the mean fluorescence intensity (MFI) and the percentage of positive cells. The mean of the standard deviation of each sample for the MFI and the percentage of positive were calculated. The results of the analysis are shown in the following chart:

Statistics

Sample	% positive		Mean fluorescence intensities	
	Mean	Std. Deviation	Mean	Std. Deviation
M1	69,9200	,08485	273,2550	1,22329
M2	56,9450	,71014	407,6000	26,6158
M3	45,5700	4,76519	196,2700	24,28602
M4	65,4850	4,26014	175,1900	4,40447
M5	64,9150	1,5547	163,1700	4,41665
M6	53,0500	,28201	214,5550	11,50479
M7	58,4300	,09340	159,0150	3,17032
M8	57,9850	,00516	180,3000	5,74234
M9	52,7500	1,56407	203,070	4,59959
M10	72,4300	,15390	123,2850	4,36554

	Average Mean	Average Std. Deviation	Average %CV
% positive	59,7480	1,3474	2,2551
IMF	209,5710	9,0329	4,3101
Valid N (listwise)	10	10	10

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e. TITLE

Title for the Immunostep CD13 PE-conjugated monoclonal antibodies was determined by performing 3 samples with different dilutions and check nonspecific staining and specific staining of normal peripheral blood from healthy donor according to the protocol shown in item 5.

Quantity (µg)	*N° of cells*	*% (in 1 x10^6 cells)*
0,125	*Up 1,73 x 10^5*	*17,3 %*
0,2	*Up 2,77 x 10^5*	*27,7 %*
0,5	*Up 6,93 x 10^5*	*69,3 %*
1	*Up 1,38 x 10^5*	*138 %*

**Note:* *Data analyzed with SPSS for Windows 21*

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