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Product: APC Anti-Human CD11b
Other names: Integrin alpha-M, ITGAM, CD11 antigen-like family member B, CR-3 alpha chain, cell surface glycoprotein MAC-1 subunit alpha, Leukocyte adhesion receptor MO1, Neutrophil adherence receptor, complement component receptor 3, Ly-40.
Cat. Ref: 11BA-100T
Reagent provided: 100 test (20µl / test)
Description: Monoclonal Mouse Anti-Human CD11b for identification of monocytes, granulocytes and NK-cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽ 0.09% sodium Azide
Clone: DCIS1/18
Immunogen: Human monocye-derived dendritic cells.
Isotype: Mouse IgG2a
Fluorochrome: APC (Ex-Max: 650 nm/Em-Max: 660). The fluorophore is excited with a dye laser (600 nm) or a helium-neon (HeNe) laser (633 nm). It is recommended to use a 660/20 band pass filter detector-equipped flow cytometer.

INTENDED PURPOSE.

CD11b APC is a monoclonal antibody that may be used to identification of monocytes, granulocytes and NK-cells.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD11b-antigen (MO-1) located on the alpha-M chain of LFA-1 complex (Lymphocyte Function-associated Antigen-1. CD11b is a member of the integrin family, primarily expressed on granulocytes, monocytes/macrophages, dendritic cells, NK cells, and subsets of T and B cells (expression is increased on activated granulocytes). CD11b non-covalently associates with CD18 (β2 integrin) to form Mac-1. Mac-1 plays an important role in cell-cell interaction by binding its ligands ICAM-1 (CD54), ICAM-2 (CD102), ICAM-4 (CD242), iC3b, and fibrinogen.

Specificity: CD11b is a integrin heterodimer of 165,95 kD glycoprotein also known as αM integrin, which comprises the receptor (CR3 chain, CD11b/ CD18) for the complement component C3i.

Integrin alpha-M/beta-2 is implicated in various adhesive interactions of monocytes, macrophages and granulocytes as well as in mediating the uptake of complement-coated particles. It is identical with CR-3, the receptor for the iC3b fragment of the third complement component. It probably recognizes the R-G-D peptide in C3b. Integrin alpha-M/beta-2 is also a receptor for fibrinogen, factor X and ICAM1. It recognizes P1 and P2 peptides of fibrinogen gamma chain.

CLINICAL RELEVANCE

The adhesion molecule CD11b, which associates with the beta2-integrin to form the Mac-1 complex, is expressed in monocytic leukemias as well as other myeloid leukemias. Its expression on the leukemic cells has been reported to correlate with more aggressive course in adult patients with AML[5]. CD11b is a therapy resistance and minimal residual disease-specific marker in precursor B-cell acute lymphoblastic leukemia[5]. CD11b expression has considerable implications for prognosis, treatment response monitoring, and MRD detection in childhood PBC-ALL.

Systemic lupus erythematosus 6 (SLEB6) is a chronic, relapsing, inflammatory, and often febrile multisystemic disorder of connective tissue, characterized principally by involvement of the skin, joints, kidneys and serosal membranes. It is of unknown etiology, but is thought to represent a failure of the regulatory mechanisms of the autoimmune system. The disease is marked by a wide range of system dysfunctions, an elevated erythrocyte sedimentation rate, and the formation of LE cells in the blood or bone marrow. Variations at the ITGAM gene, which encodes for the CD11b chain of the Mac-1 (alphaMbeta2; CD11b/CD18; complement receptor-3) integrin, is one of the strongest genetic risk factors for systemic lupus erythematosus (SLE)[3].

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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.

PRINCIPLES OF THE TEST.

Immunostep CD11b APC monoclonal antibodies bind to the surface of cells that express the CD11b-antigen. To identify these cells, peripheral blood leucocytes are incubated with the reagent. Analysed by flow cytometry with 633 nm helium-neon (HeNe) laser. It is recommended to use a 660/20 nm band pass filter detector-equipped flow cytometer.

REAGENTS.

Cluster Designation:	Anti-Human CD11b
Clone:	DCIS1/18
Isotype:	IgG2a, kappa
Composition:	IgG2a heavy chain Kappa light chain
Species:	Mouse
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	APC Excitation wavelength 633 nm Emission wavelength 660 nm
Molar composition:	APC/protein ± 1 (0,5-1,5)
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.

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- 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.

- 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
- The normal appearance of the APC conjugated monoclonal antibody is a blue clear liquid without aggregates or precipitates and completely odourless.

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 CD11b APC 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 APC-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**

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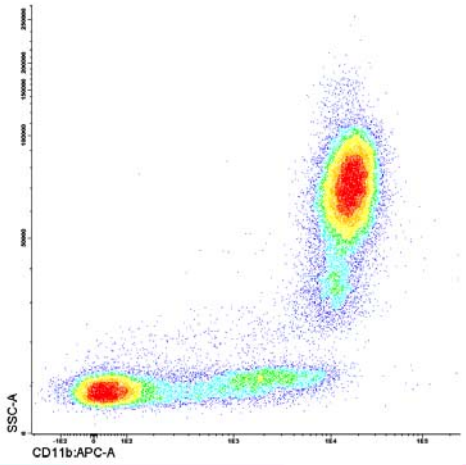
6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents:	Mouse IgG2a: APC
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, 7-AAD or propidium iodide, 0.25% (w/v) in PBS for the determination of cell viability	Optional
Lysing Solution	
Fixing Solution	
Flow cytometer:	The equipment must be equipped with a 633 nm helium-neon (HeNe) laser. It is recommended to use a 660/20 nm band pass filter 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 APC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained 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. 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 a biparametric representation (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on leucocytes. Human peripheral blood lymphocytes were stained with CD11b APC and CD45 PerCP. Cells were analyzed on a FACSAria II (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

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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.

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. 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

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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	

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*

CD11b is expressed on granulocytes, monocytes/macrophages, dendritic cells, NK cells, and subsets of T and B cells. CD11b expression is increased on activated granulocytes.

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 CD11b APC monoclonal antibody. Non-specific fluorescence identified by the APC conjugated isotypic control IgG2a was analysed. Cells contained Platelets, B Lymphocytes and Erythrocytes in CD11b positive region 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:

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Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
% Isotype control	10	,21	1,18	,3870	,28721
% Erythrocytes	10	,08	,40	,2030	,11046
% Platelets	10	,18	,98	,3930	,28277
% B Lymphocytes	10	,26	1,70	,7870	,49924
Valid N (listwise)	10				

b. SENSIBILITY or LINEARITY

Sensitivity of the Immunostep CD11b APC monoclonal antibody was determined by staining U937 cell line as positive population and Nalm-6 cell line as negative population. 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.

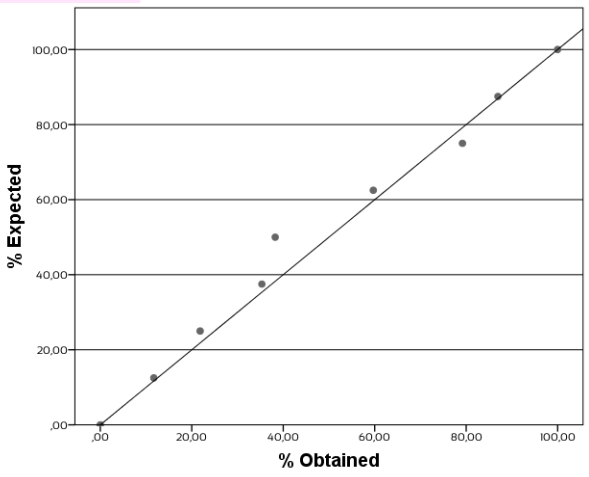
To determine the consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use.

Model Summary[b]

R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
,993	,985	,983	4,52684	y = 1.020x – 2,882

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. CD11b APC sensibility was demonstrated from 1 x 10^5 to 1 x 10^6 cells in 1 x 10^6 total cells.

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c. REPRODUCIBILITY

Reproducibility for the Immunostep CD11b APC-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 CD11b+ 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

Percentage	Mean	Std. Deviation	Minimum	Maximum
High	77,8050	,95003	76,20	79,23
Medium	75,4330	,78103	74,35	77,11
Low	74,4510	,73703	73,45	75,93

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 average mean and the average standard deviation of MFI and percentage of positive cells were calculated. Monocytes and neutrophils CD11b +/CD45 cells were selected in the analysis.

The results of the analysis are shown in the following chart:

	Average Mean	Average Std. Deviation	Average %CV
% positive	72,8929	0,4608	0,6321
IMF	27685,80	3151,9130	11,3845
Valid N (listwise)	10	10	10

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

As shown in the table, the results show excellent repeatability from batch to batch, especially for calculating the percentage of positive cells in which the value is far below 10%

12. BIBLIOGRAPHY.

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