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Product: PE Anti-human CD21
Other names: CR2/CR1, CRB1, C3DR
Cat. Ref: 21PE-100T
Reagent provided: 100 test (20µl / test)
Description: Monoclonal Mouse Anti-Human CD21 PE is recommended for use in flow cytometry for identification of B cells subsets in peripheral blood. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide
Clone: HI21a
Isotype: IgG2a
Fluorochrome: R-Phycoerythrin (R-PE). PE (Ex.: 496, 564 nm/Em-Max: 578 nm). Recommended 488, 532 or 561 laser, 556 LP filter and 585/42 or 575/26 detector-equipped flow cytometer.

INTENDED PURPOSE.

CD21 PE is a monoclonal antibody conjugated that may be used to identification of B cells subsets in peripheral blood.

TECHNICAL SUMMARY.

Reactivity: HI21a monoclonal antibody reacts with human CD21, an approximately 145 kDa type I transmembrane protein. CD21, also known as Complement component (3d / Epstein Barr virus) receptor 2 and CR2, is a member of the CD system and is a protein involved in complement system.

CD21 is expressed by mature B cells, folliculate dendritic cells and a subset of epithelial cells and is a receptor for complement component C3d and Epstein-Barr virus (EBV). CD21, in association with CD19 and CD81, participates in the multimolecular complex with BCR and is involved in B cell activation. CD21 on mature B-cells form a complex called the B cell receptor complex with two other membrane proteins, CD19 and CD81. CD21 has a function in the complement system through serving as the cellular receper specific for ligands such as C3 and C4 which can be attached to foreign macromolecules in order to remove or uptake them. This results in B-cells having enhanced response to the antigen.

CLINICAL RELEVANCE

Differential expression of CD21 identifies developmentally and functionally distinct subsets of human transitional B cells. This finding provide important insights into the process of human B-cell development and have implicatons for understanding the processes underlying perturbed B-cell maturation in autoimmune and immunodeficient conditions.

Recent publications have demonstrated that T cell subsets expressing CD21 and CD32 may differ with respect to the presence or clinical forms of multiple sclerosis disease.

PRINCIPLES OF THE TEST.

Immunostep CD21 PE monoclonal antibodies bind to the surface of cells that express the CD21 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:	Anti-Human CD21
Clone:	HI21a
Isotype:	IgG2a
Species:	Mouse
Composition:	IgG2a heavy chain

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	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 (0,5-1,5)
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests, containing 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-Phycoeritryn (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 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.

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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 CD21 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 (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.

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents:	Mouse IgG2a: PE
Leucocyte gating reagent:	Mouse anti-human CD45: FITC
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 or 561-nm (Yellow-Green) laser, 556 LP filter, 585/42 or 575/26 detector-equipped and appropriate computer hardware and software.

7. INTERPRETATION OF RESULTS.

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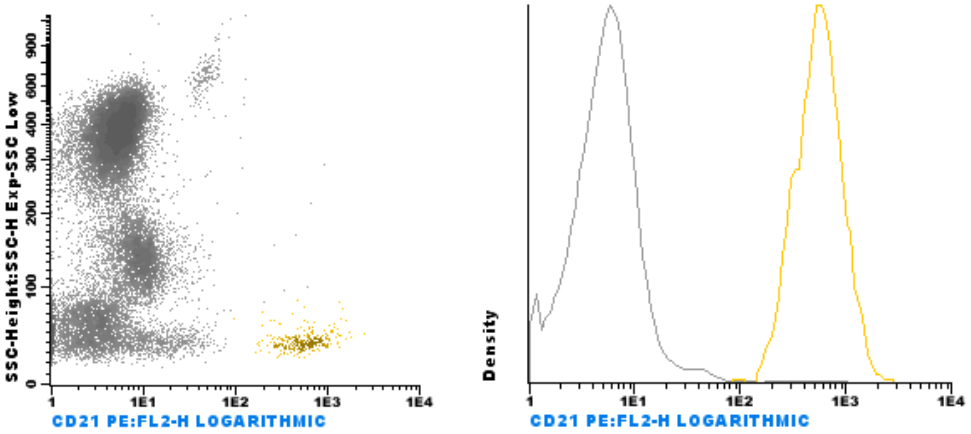
a. *FLOW CYTOMETRY*

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.



The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on leukocytes. Human peripheral blood lymphocytes were stained with CD21 PE and CD45 PerCP. Lymphocytes CD21+ are represented by the yellow histogram. 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 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.

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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
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		4.3 - 10.0 $X10^3$/µL

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(WBC)		
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. Samples were stained with Immunostep CD21 PE monoclonal antibody. Non-specific fluorescence identified by the PE conjugated isotypic control was analysed. Cells contained in the lymphocyte, monocyte, granulocyte, platelets and erythrocytes regions were selected for analysis with the appropriate markers. Blood samples were processed by a Staining Cell Surface Antigens for Flow Cytometry Protocol for flow cytometric analysis.

The results obtained are shown in the following table:

Statistics

		% Isotype control	% T lymphocytes	% Monocytes	% Granulocytes	% Platelets	% Erythrocytes
N	Valid	10	10	10	10	10	10
	Missing	0	0	0	0	0	0
Mean		,1120	,0830	,0090	,0000	,0080	,0050
Median		,0700	,0700	,0050	,0000	,0000	,0050
Std. Deviation		,13677	,04990	,01524	,00000	,01874	,00527
Variance		,019	,002	,000	,000	,000	,000
Range		,43	,15	,05	,00	,06	,01
Minimum		,01	,02	,00	,00	,00	,00
Maximum		,44	,17	,05	,00	,06	,01

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, granulocytes, platelets and erythrocytes stained with the mentioned MAb was evaluated.

The results show high specificity for its population and minimal unspecificity appearing in other populations.

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b. SENSIBILITY or LINEARITY

Sensitivity of the Immunostep CD21 PE 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

Model Summary[b]

R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
,992[a]	,984	,982	,13870	y = 0,9325x – 0,0313

a. Predictors: (Constant), % Expected

b. Dependent Variable: % Obtained

c. REPRODUCIBILITY

Reproducibility for the Immunostep CD21 PE-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each sample in each of three leukocyte ranges: high, medium and low. Three samples of each range were used. Thus, a total of 30 determinations were performed for each type of percentage. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 30 determinations for each range were performed by the staining, processing and analysis of 3 separate samples. Lymphocytes CD21+ were selected for the analysis of percent cells stained in each measure.

To perform this study, anticoagulated blood was obtained from normal donors expressing a different percentage of leukocytes.

Low percentage of leukocytes

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Sample 1	10	4,96	5,50	5,3020	,15098	,023
Sample 2	10	2,31	2,75	2,5360	,14886	,022
Sample 3	10	2,06	2,51	2,2920	,13750	,019
Valid N (listwise)	10					

High percentage of leukocytes

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Sample 1	10	2,97	3,89	3,3690	,29558	,087
Sample 2	10	1,54	1,95	1,7690	,12662	,016
Sample 3	10	4,26	4,88	4,5230	,20238	,041
Valid N (listwise)	10					

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Normal percentage of leukocytes

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Sample 1	10	2,23	2,81	2,6890	,16802	,028
Sample 2	10	4,02	4,44	4,2230	,11954	,014
Sample 3	10	3,21	3,93	3,5480	,22464	,050
Valid N (listwise)	10					

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

12. BIBLIOGRAPHY.

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