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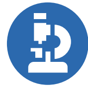
Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

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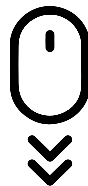

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Product: FITC Anti-Human CD21
Other names: Complement receptor type 2 (Cr2), Complement C3d receptor, Epstein-Barr virus receptor (EBV receptor), *C3DR, CR2/CR1, CRB1, C3DR,*

Cat. Ref: 21F-100T
Reagent provided: 100 test (20µl / test*)
Description: Monoclonal Mouse Anti-Human CD21 FITC 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
Immunogen: Tonsil cells.
Fluorochrome: FITC (Ex-Max: 494 nm/Em-Max: 520 nm). The fluorophore is excited with the blue laser (488 nm) ion argon laser. It is recommended to use a 502 LP dichroic mirror and 530/30 band pass filter detector-equipped flow cytometer.
* see Title below.

INTENDED PURPOSE.

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

TECHNICAL SUMMARY.

Reactivity: Clone HI21a reacts with the human form of the 145 kDa glycosylated type I integral membrane protein. It is a receptor for the C3d complement fragment and for Epstein Barr virus (EBV), found on mature B cells, follicular dendritic cells, andsome epithelial cells. It is also weakly expressed on a subset of mature T cells (a subset of CD3 + cells is also CD21) and thymocytes. Human CD21 is a receptor for complement fragments C3d, C3dg or ic3D and also EBV. CD21 is also a ligand for CD23 and plays a role in IgE synthesis.

This clone also cross-reacts with a major subset of, but not all, peripheral blood CD20 + lymphocytes of baboon, and both rhesus and cynomolgus macaque monkeys.

Specificity: The monoclonal antibody is directed against the CD21-antigen, which is expressed on normal Ig-positive B-cells from peripheral blood and lymphoid tissues and on dendritic cells of germinal centres. Its expression is lost on activated B-cells. The distinct distribution among B-cell malignancies differs from other B-cell markers e.g. CD19 and CD20.

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 implications 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 FITC 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 Argon laser at 488 nm.

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REAGENTS.	
Cluster Designation:	CD21
Clone:	HI21a
Isotype:	IgG2a
Species:	Mouse
Composition:	IgG2a heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	Fluorescein isothiocyanate Excitation wavelength 488 nm Emission wavelength 520 nm
Molar composition:	FITC/protein 6,0 – 8,0
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤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. APPROPIATE STORAGE CONDITIONS.

- Fluorescein (FITC): keep in dark place at 2-8°C. DO NOT FREEZE.

**Note: it's been described stored conjugated monoclonal antibodies on FITC at -20°C. This can affect to the conjugated intense.*

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 FITC conjugated monoclonal antibody is a clear yellow-orange 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.

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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.

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 CD21 FITC-conjugated monoclonal antibody and 180µl of phosphate buffered saline (PBS) to tubes marked unknown. In other control tube add 10µl of corresponding Immunostep IgG2a FITC-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: FITC

Leucocyte gating reagent: Mouse anti-human CD45: APC/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

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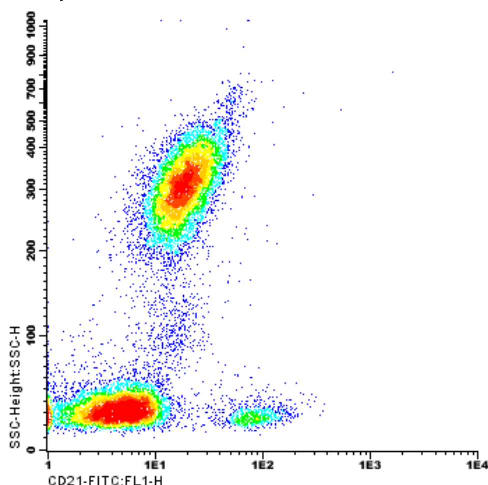
Flow cytometer: Becton Dickinson FACScan™, Coulter Profile or equivalent.

7. INTERPRETATION OF RESULTS.

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 FITC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained B lymphocytes is determined. An appropriate FITC-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.



The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate mouse whole blood sample. Mouse peripheral blood lymphocytes were stained with CD21 FITC 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-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. 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 $X10^3/\mu L$
Lymphocytes*	25 – 33 %	1.0 - 4.8 $X10^3/\mu L$
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 $X10^3/\mu L$
Eosinophils	1 – 3 %	0.05 – 0,25 $X10^3/\mu L$
Basophils	0 – 0,075 %	0,015 – 0,05 $X10^3/\mu L$
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.

SPECIFICITY

CD21is expressed on B lymphocytes cells. 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 CD21 FITC monoclonal antibody. Non-specific fluorescence identified by the FITC-conjugated isotypic control IgG2a was analysed. Cells contained in platelets, erythrocytes, monocytes and T lymphocyte regions were selected for analysis. Blood samples were processed by a Staining Cell Surface Antigens for Flow Cytometry Protocol described in Section 5.

The results obtained are shown in the following table:

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
% Isotype control	10	,02	,85	,3110	,27307
% Platelets	10	,04	2,84	,5430	,83375
% Erythrocytes	10	,00	,09	,0360	,02914
% T Lymphocyte	10	5,05	32,16	17,6330	7,88625
% Monocytes	10	,00	,33	,1800	,11363
% B Lymphocytes	10	1,04	6,19	2,4250	1,73294
Valid N (listwise)	10				

LINEARITY OF QUANTITATIVE MEASUREMENT

Linearity of the Immunostep CD21 FITC monoclonal antibody was determined making dilutions between Ramos cell line and Jurkat cell line in known proportion. Cells were mixed in different proportions with a constant final number of 1×10^6 cells to achieve different cell ratios from 0% positive cells to 100%. It provides an indication of its reliability during its normal use.

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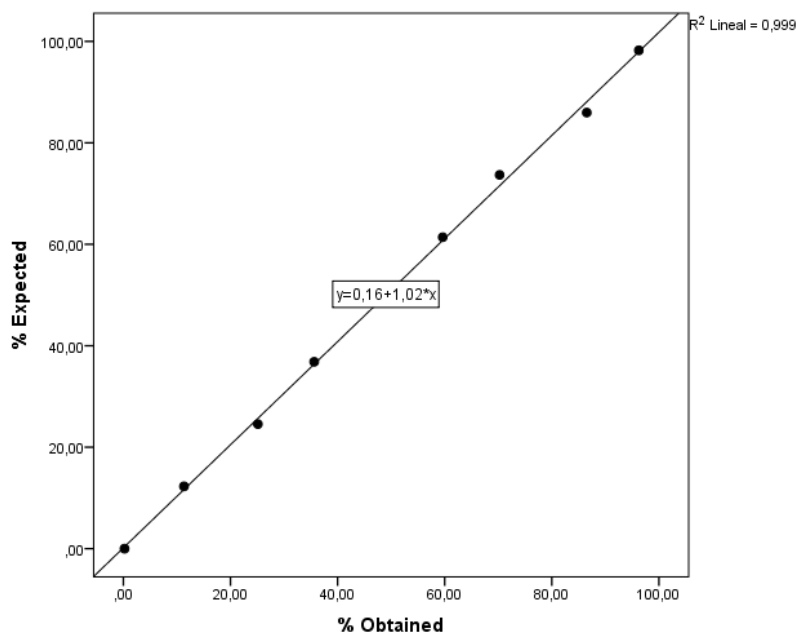
Thereafter cells were incubated with the antibody to the recommended amount for 15 minutes. Finally the cells were washed according to standard protocol. A linear regression between the expected values and the observed values was calculated. The results obtained are summarized in the following table:

Model Summary[b]

R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
0,999[a]	0,999	0,999	0,999	y =1,016x + 0,158

a. Dependent Variable: % Expected

b. Predictors: (Constant) % Obtained



The results show an excellent correlation between the results obtained and expected based on the dilution used. CD21 FITC linearity was demonstrated from 1 x 10^5 to 1 x 10^6 cells in 1 x 10^6 total cells.

REPRODUCIBILITY

Reproducibility for the Immunostep CD21 FITC-conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three ranges of lymphocytes; high, medium and low. Thus, a total of 30 determinations were performed. 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 CD21+ were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from three different donors expressing a high, medium and low percentage of Lymphocytes.

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Descriptive Statistics

Range		N	Minimum	Maximum	Mean	Std. Deviation
High	Porcentage	10	28,24	30,92	29,5800	,68882
	IMF	10	1695,00	1762,00	1726,5000	20,12875
	Valid N (listwise)	10				
Medium	Porcentage	10	23,74	27,89	24,7440	1,15171
	IMF	10	1631,00	1672,00	1644,1000	14,01150
	Valid N (listwise)	10				
Low	Porcentage	10	9,75	10,30	10,0480	,19418
	IMF	10	1285,00	1385,00	1342,1000	25,79599
	Valid N (listwise)	10				

12. BIBLIOGRAPHY.

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