

# Anti-Human CD21 (HI21a)

Fluorochrome	Reference	Test
FITC	21F-100T	100 test
PE	21PE-100T	100 test



## PRODUCT DESCRIPTION

**Other Names:** Complement receptor type 2 (Cr2), Complement C3d receptor, Epstein-Barr virus receptor (EBV receptor), C3DR, CR2/CR1, CRB1, C3DR,  
**Description:** The anti-CD21 monoclonal antibody derives from Tonsil cells

**Clone:** HI21a

**Isotype:** Mouse IgG2a, kappa

**Reactivity:** Human

**Source:** Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.

**Purification:** Affinity chromatography.

**Compositión:** Mouse anti-human CD21 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN<sub>3</sub>).

Fluorochrome	Reagent provided	Concentration (µg/ml)
FITC (Fluorescein isothiocyanate)	150 ug in 2 ml	75
PE (R-Phycoerythrin)	25 ug in 2 ml	12,5

## RECOMMENDED USAGE

Immunostep's CD21, clone HI21a, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD21 using flow cytometry.

## 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.<sup>(1-4)</sup>

## PRINCIPLES OF THE TEST

The anti-CD21 monoclonal antibody binds to the surface of cells that express the CD21 antigen. To identify these cells, the sample is incubated with the antibody and is analysed by flow cytometry.

## APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the date indicated.

Once the vial is open, the product is stable for 90 days.

## EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: [tech@immunostep.com](mailto:tech@immunostep.com)

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

## RECOMMENDATIONS AND WARNINGS

- The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at [www.immunostep.com](http://www.immunostep.com)
- Avoid microbial contamination of the reagent.
- Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
- Never mouth pipette.
- In the case of contact with skin, wash in plenty of water.
- The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- FOR *IN VITRO* DIAGNOSTIC USE.
- For professional use only.
- Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

## SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)<sup>5,6</sup>. For optimum results, the sample should be processed during the six hours following the extraction. Samples which cannot be processed within the 48 hours following the extraction should be discarded.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Isotype controls:

Fluorochrome	Isotype control	Immunostep Reference
FITC	Mouse IgG2a	ICIGG2AF-100UG
PE	Mouse IgG2a	ICIGG2APE-50UG

- Centrifuge
- Commonly used 12 x 75-mm flow cytometry assay tubes
- Micropipettes for dispensing volumes from 5 µl to 2 ml
- Blood collection tubes with anticoagulant.
- Phosphate buffered saline (PBS) with 0.09% sodium azide. It is recommendable to add 0.5% BSA
- Vacuum system
- Lysing solution
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

**SAMPLE PREPARATION:**

1. Add the suggested volume indicated on the antibody vial to a 12x75-mm cytometer tube. It is advisable to prepare an additional tube with the appropriate isotype control (*please see materials required but not provided*).
2. Add 100 µL of sample (up to 10<sup>6</sup> cells) and mix properly in the vortex.
3. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
4. Add 2 ml of the lysing solution, mix in the vortex and incubate in the dark for 10 minutes or until the sample is lysed.
5. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
6. Resuspend pellet.
7. Add 2 ml of PBS (*please see materials required but not provided*).
8. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
9. Resuspend the pellet in 0.3 ml of PBS.

Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out. Samples should be acquired within the 3 hour after lysis.

**FLOW CYTOMETRY ANALYSIS**

Collect the fluorescence attributed to monoclonal antibody CD21 and determine the percentage of stained cells.

It is necessary to use an isotype control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD21, so as to evaluate and correct the unspecific binding of leucocytes (*please see materials required but not provided*). Set an analysis region to eliminate fluorescence background noise and to include positively stained cells.

Below is an example diagram of stained cells:

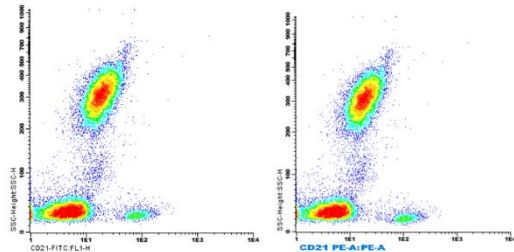


Fig. 1: Biparametric diagram of the average fluorescence intensity of peripheral blood sample stained with CD21+ and its internal complexity (SSC).

**LIMITATIONS OF THE PROCEDURE**

1. Incubation of antibody with cells for other than the recommended procedures may result in a reduction or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.
3. Abnormal cells or cell lines may show a higher antigen density than normal cells. In some cases, this could require the use of a greater quantity of monoclonal antibody than is indicated in the procedures for sample preparation.
4. In whole blood samples, red blood cells found in abnormal samples, as well as nucleated red cells (from both normal and abnormal specimens) may be resistant to lysis. Longer periods of red blood cell lysing may be needed in order to avoid the inclusion of unlysed cells in the lymphocyte gated region.
5. Blood samples should not be refrigerated for an extensive period (more than 24 hours), since the number of viable cells will gradually decrease, and this may have an effect on the analysis. In order to obtain the best values, they should be kept at room temperature immediately prior to incubation with the monoclonal antibody.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

**REFERENCE VALUES**

Abnormal results in the percentage of cells expressing the antigen or in its levels of expression may be due to pathological conditions. It is advisable to know the normal antigen expression patterns in order to ensure a proper interpretation of the result<sup>7,8,9</sup>

The values obtained from healthy individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.

**CHARACTERISTICS**

SPECIFICITY

CD21 is 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

monoclonal antibody. Non-specific fluorescence identified by the conjugated isotype 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.

The results obtained are shown in the following table:

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
% Isotype control	10	0,02	0,85	0,3110	0,27307
% Platelets	10	0,04	2,84	0,5430	0,83375
% Erythrocytes	10	0,00	0,09	0,0360	0,02914
% T Lymphocyte	10	5,05	32,16	17,6330	7,88625
% Monocytes	10	0,00	0,33	0,1800	0,11363
Valid N (listwise)	10				
% Isotype control	10	0,01	0,44	0,112	0,13677
% Platelets	10	0	0,06	0,008	0,01874
% Erythrocytes	10	0	0,01	0,005	0,00527
% T Lymphocyte	10	0,02	0,17	0,083	0,0499
% Monocytes	10	0	0,05	0,009	0,01524
Valid N (listwise)	10				

#### SENSIBILITY

Linearity of the Immunostep CD21 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  $1 \times 10^6$  cells to achieve different cell ratios from 0% positive cells to 100%. It provides an indication of its reliability during its normal use.

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 <sup>b</sup>				
R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
FITC				
0,999 <sup>a</sup>	0,999	0,999	0,999	$y = 1,016x + 0,158$
PE				
0,992 <sup>a</sup>	0,984	,982	,13870	$y = 0,9325x - 0,0313$

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 linearity was demonstrated from  $1 \times 10^5$  to  $1 \times 10^6$  cells in  $1 \times 10^6$  total cells.

#### REPRODUCIBILITY

Reproducibility for the Immunostep CD21 -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.

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

\*Note: Data analyzed with SPSS for Windows 21

#### WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

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