Anti-Human CD19 (A3-B1)

Fluorochrome	Reference	Test
FITC	19F1-100T	100 test
PE	19PE1-100T	100 test
APC	19A1-100T	100 test

IVD CE immunostep we know how

PRODUCT DESCRIPTION

Other Names: B-lymphocyte antigen CD19, B-lymphocyte surface antigen B4, Differentiation antigen CD19, T-cell surface antigen Leu-12, B4
Description: The anti-CD19 monoclonal antibody derives from human tonsil.
Clone: A3-B1
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 CD19 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN_3).

Fluorochrome	Reagent provided	Concentration (µg/ml)
FITC (Fluorescein isothiocyanate)	50 ug in 2 ml	25
PE (R-Phycoerythrin)	50 ug in 2 ml	25
APC (Allophycocyanin)	30 ug in 2 ml	15

RECOMMENDED USAGE

Immunostep's CD19, clone A3-B1, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD19 using flow cytometry.

CLINICAL RELEVANCE

The CD19 monoclonal antibody may be used to identify and enumerate CD19+ B lymphocytes in human peripheral blood. This may be valuable, in combination with other indicators, for the diagnosis or prognosis of some immunodeficiency diseases, including agammaglobulinemia, severe combined immunodeficiency disease (SCID) and common variable immunodeficiency disease (CVID) which are all reported to demonstrate reduced numbers of circulating B lymphocytes. The reagent may also be of value in determining the lineage of malignant lymphoid cells in cases of chronic and acute leukaemia and lymphoma, with the great majority of B cell malignancies expressing CD19[1-3].

PRINCIPLES OF THE TEST

The anti-CD19 monoclonal antibody binds to the surface of cells that express the CD19 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

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 ⚠

a) 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
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR *IN VITRO* DIAGNOSTIC USE.
j) For professional use only.
k) 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)[4,5]. 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		ICIGG2APE-50UG
APC		ICIGG2AA-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 10^6 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 CD19 and determine the percentage of stainend 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 CD19, 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:

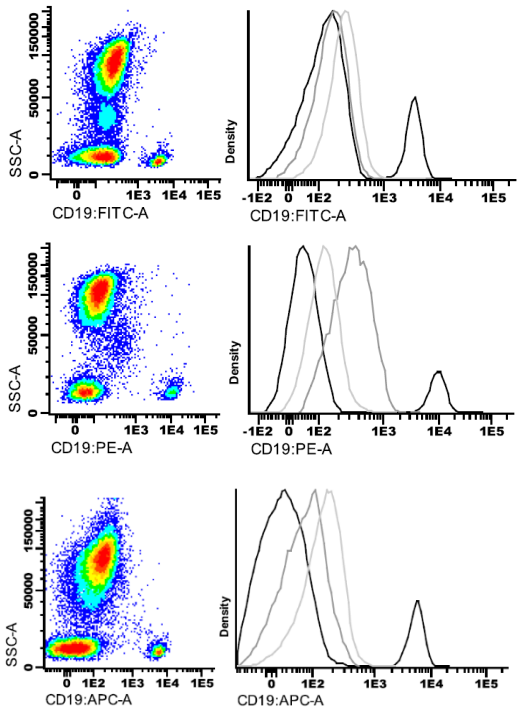

Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of peripheral blood stained with CD19+ and its internal complexity (SSC). Right, a diagram of the same specimen in histogram format.

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
6. 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 results[6,7,8]

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

CD19 is expressed from the earliest stages of B-progenitor development and on all peripheral B cells including germinal centre B cells, all B cell lines tested and B cell leukaemias tested.
The antigen is lost on B cell maturation to plasma cells.

CD19 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 CD19 monoclonal antibody.
Non-specific fluorescence identified by an 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.

			Lymphocytes	Monocytes	Granulocytes
FITC	N	Valid	5	5	5
		Missing	0	0	0
	Mean		5,49	6,24	4,22
	Median		5,18	7,12	4,29
	Mode		1,69 (a)	3,71(a)	1,94(a)
	Std. Deviation		3,94	2,32	1,93
	Variance		15,52	5,41	3,75
	Range		8,79	5,02	5,14

			Lymphocytes	Monocytes	Granulocytes
PE	N	Valid	10	10	10
		Missing	0	0	0
	Mean		48,38	97,37	99,58
	Median		47,61	98,28	99,95
	Mode		30,67 (a)	88,95 (a)	99,99 (a)
	Std. Deviation		11,58	3,25	0,78
	Variance		134,23	10,57	0,61
	Range		35,34	11,00	2,33
			Lymphocytes	Monocytes	Granulocytes
APC	N	Valid	10	10	10
		Missing	0	0	0
	Mean		13,43	8,81	0,38
	Median		11,16	7,63	0,35
	Mode		2,26(a)	3,13(a)	0,29(a)
	Std. Deviation		10,30	5,20	0,11
	Variance		106,29	27,09	0,012
	Range		33,74	14,97	0,31

SENSIBILITY

Sensitivity of the Immunostep CD19 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	R	R Square	Adjusted R Spuare	Std. Error of the Estimate
FITC	0,983(a)	0,967	0,962	1,10648
PE	0,997 (a)	0,994	0,993	0,17612
APC	0,992(a)	0,984	0,982	0,08903

REPRODUCIBILITY

Reproducibility for the Immunostep CD19 -conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three CD19+ ranges, high, medium and low. Thus, a total of 30 determinations were performed for each form of CD19.
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 were selected for the analysis of percent cells stained in each of the three ranges.
To perform this study, anticoagulated blood was obtained from a normal donor expressing a high percentage of CD19+ cells. Mid-range and low range samples were obtained by mixing known CD19- cells in appropriate ratios, while maintaining the same total cell concentration for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples.

FITC	N	Minimum	Maximum	Mean	Std. Deviation
High	10	12,70	13,63	13,14	0,29
Medium	10	6,92	7,76	7,44	0,27
Low	10	2,00	3,24	2,48	0,32
Valid N (listwise)	10				
PE	N	Minimum	Maximum	Mean	Std. Deviation
High	10	13,95	14,40	14,10	0,12
Medium	10	5,40	5,89	5,66	0,12
Low	10	0,33	0,77	0,52	0,11
Valid N (listwise)	10				
APC	N	Minimum	Maximum	Mean	Std. Deviation
High	10	14,17	14,84	14,39	0,19
Medium	10	4,37	4,84	4,58	0,16
Low	10	0,57	0,95	0,70	0,10
Valid N (listwise)	10				

WITHIN-LABORATORY PRECISION (INTRA-ASSAY)

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. Lymphocytes CD19+/CD45+ cells were selected in the analysis.

		Average Mean	Average Std. Deviation	Average %CV
PE	% positive	1,81	0,09	5,50
	IMF	8912,20	198,49	2,22
	Valid N (listwise)	10	10	10
FITC	% positive	3,77	0,14	3,87
	IMF	3953,50	108,24	2,73
	Valid N (listwise)	10	10	10

As shown in the table, the results show excellent repeatability from lot to lot, both average %CV percentages of positive cells and MFI as show values.

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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MANUFACTURED BY

Immunostep S.L
Avda. Universidad de Coimbra, s/n
Cancer Research Center (CIC)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. (+34) 923 294 827
www.immunostep.com