

# Anti-Human CD3 – CD19 (33-2A3/A3-B1)

Fluorochrome	Reference	Test
FITC/PE	3FI19PEI-50T	50 tests



## PRODUCT DESCRIPTION

**Description:** The anti-CD3/CD19 monoclonal antibody is derived from human leukocytes (CD3) and tonsil cells (CD19).

**Clone:** 33-2A3, A3-B1

**HLDA:** CD3 2<sup>nd</sup> International Workshop on Human Leukocyte Differentiation.

**Isotype:** Mouse IgG2a, kappa

**Reactivity:** Human

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

**Purification:** Affinity chromatography.

**Composition:** Fluorochrome-conjugated mouse anti-CD3/CD19 monoclonal antibody in an aqueous solution containing stabilising protein and 0.09% sodium azide (NaN<sub>3</sub>).

Fluorochrome	Reagent supplied	Concentration (µg/ml)
FITC (fluorescein isothiocyanate)	20 µg in 2 ml	10
PE (Rhodamine)	10 µg in 2 ml	15

## RECOMMENDED USE

Immunostep CD3/CD19, clones 33-2A3 and A3-B1, is a monoclonal antibody intended for in vitro diagnostic use for the identification and enumeration of lymphocytes in human samples expressing CD3 and CD19 using flow cytometry.

## CLINICAL SIGNIFICANCE

CD3 is used for the diagnosis of certain diseases that cause a decrease in the number of immunocompetent cells, generally a decrease in the number of mature CD3+ T lymphocytes.

CD19 can be used for the diagnosis or prognosis of certain immunodeficiency diseases related to CD19+ B lymphocytes. The reagent may also be valuable in determining the lineage of malignant lymphoid cells in cases of chronic and acute leukaemia and lymphoma, with the vast majority of malignant B-cell tumours expressing CD19<sup>(7-9)</sup>.

## TEST PRINCIPLES

The anti-CD3/CD19 monoclonal antibody binds to the surface of cells expressing the CD3 and CD19 antigens. To identify these cells, the sample is incubated with the antibody and analysed in a flow cytometer.

## CONDITIONS STORAGE AND HANDLING

Store in darkness, refrigerated between 2 and 8 °C. DO NOT FREEZE. The antibody is stable until the date shown on the vial label if stored between 2 and 8 °C. Do not use after this date.

Once the vial has been opened, the product is stable for a period of 90 days.

## SIGNS OF DETERIORATION

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

The normal appearance is that of a semi-transparent, odourless liquid. There should be no precipitates or cloudiness. There should be no odour.

## RECOMMENDATIONS AND WARNINGS



- The reagents contain sodium azide. Under acidic conditions, it is converted to hydrazonic acid, an extremely toxic compound. Azide compounds should be dissolved in tap water before disposal. These conditions are recommended to avoid deposits in pipes, where explosive conditions could develop. Safety data sheet (SDS) available at the [www.immunostep.com](http://www.immunostep.com)
- Avoid microbial contamination of the reagent.
- Avoid exposure to light. Use dim light during handling, incubation with cells, and before analysis.
- Do not pipette by mouth.
- In case of skin contact, wash with plenty of water.
- Samples should be treated in the same way as those that could transmit infections. Appropriate methods for handling them should be available.
- Do not use after the expiry date stated on the vial.
- Deviations from the recommended procedures could invalidate the test results.
- FOR IN VITRO DIAGNOSTIC USE
- For professional use only.
- Before acquiring samples, verify that the flow cytometer is calibrated and compensated.

## SAMPLE COLLECTION

Venous blood samples should be collected in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)<sup>1,2,3</sup>. For optimal results, the sample should be processed within 6 hours of collection. Samples that cannot be processed within 48 hours of collection should be discarded.

## REQUIRED MATERIALS NOT SUPPLIED

- Isotype controls:

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

- Centrifuge
- Standard 12 x 75 mm test tubes for flow cytometry
- Micropipettes capable of dispensing volumes between 5 µl and 2 ml.
- Blood collection tubes with anticoagulant.
- Phosphate-buffered saline (PBS) with 0.09% sodium azide. It is recommended to add 0.5% BSA.
- StepCount – 25 tests (Ref: 139999121). Reagent available for single-platform cell counting, which can be used in combination with this reagent.
- Vacuum system
- Lysis solution
- Flow cytometer equipped with laser and filters suitable for the fluorochrome.
- Vortex mixer

## SAMPLE PREPARATION:

### Lysis protocol with washes:

The reagent is designed for protocols with and without washing after lysis. The latter case is common in cell counting protocols.

1. Add the recommended volume in the antibody vial to a 12x75 mm flow cytometry tube. It is advisable to prepare an additional tube with the appropriate isotype control (*see materials required but not supplied*).
2. Add 100 µL of sample (up to  $10^6$  cells) and mix thoroughly in the vortex.
3. Incubate in the dark at room temperature (20-25°C) for 15 minutes or at 4°C for 30 minutes.
4. Add 2 ml of the lysis solution, vortex and incubate in the dark for 10 minutes or until the sample is lysed.
5. Centrifuge at 540g for 5 minutes and aspirate the supernatant, taking care not to touch the cell pellet. Leave about 50 µl of liquid un aspirated.
6. Resuspend the pellet.
7. Add 2 ml of PBS (*see materials required but not supplied*).
8. Centrifuge at 540g for 5 minutes and aspirate the supernatant, taking care not to disturb the cell pellet. Leave approximately 50 µl of liquid un aspirated.
9. Resuspend the pellet in 0.3 ml of PBS.

Acquire in a flow cytometer or store at 2-8°C in the dark until analysis. Samples must be acquired within 3 hours of lysis.

### Lysis protocol without washing:

1. Add the recommended volume of conjugated monoclonal antibody to a 12 x 75 mm flow cytometry tube. It is advisable to prepare an additional tube with

the appropriate isotype control (see materials required but not supplied).

2. Add 50 µL of well-homogenised sample and mix thoroughly in the vortex.
3. Incubate in the dark at room temperature (20-25°C) for 15 minutes or at 4°C for 30 minutes.
4. Add 450 µl of lysis solution to the tube. Vortex and incubate in the dark for 10 minutes (or until the sample clarifies, indicating complete lysis of the erythrocytes).

The sample is now ready for analysis by flow cytometry.

## ANALYSIS BY FLOW CYTOMETRY

Collect the fluorescence attributed to the CD3/CD19 monoclonal antibody and determine the percentage of labelled cells. An isotype control conjugated with the same fluorochrome, immunoglobulin heavy chain type and concentration as CD3/CD19 should be used to estimate and correct for non-specific binding of lymphocytes (*see materials required but not supplied*), generate an analysis region to eliminate fluorescence background noise and include correctly labelled cells.

Below is an example of the representation of labelling in peripheral blood from a healthy donor following the protocol described in point 6.

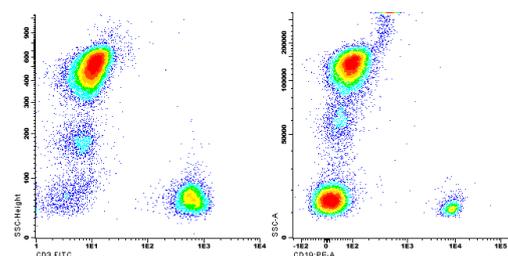


Fig. 1: Biparametric diagram of the mean fluorescence intensity of CD3+ and CD19+ lymphocytes and their internal complexity (SSC) in normal peripheral blood from a healthy patient.

## LIMITATIONS OF THE PROCEDURE

1. Incubating the antibody with the cells without following the recommended procedures may result in a decrease or loss of the antigenic determinants on the cell surface.
2. Values obtained from normal individuals may vary between laboratories; therefore, it is recommended that each laboratory establish its own normal ranges.
3. Abnormal cells or cell lines may show higher antigen density than normal cells. This may require, in some cases, the use of a greater amount of monoclonal antibody than indicated in the sample preparation procedures.
4. In whole blood samples, erythrocytes found in pathological samples, as well as nucleated red blood cells (from both normal and pathological samples), may be resistant to lysis. Longer erythrocyte lysis times may be required to avoid the inclusion of unlysed cells in the leukocyte region.
5. Blood samples should not be refrigerated for an excessive period (more than 24 hours), as the number of viable cells will decrease over time and may even interfere with the analysis. For best results, they should be kept

- at room temperature for a few minutes before incubation with the monoclonal antibody.
6. The most accurate results with flow cytometry procedures depend on correct alignment and calibration of the lasers, as well as the establishment of the correct regions.

**REFERENCE VALUES**

Abnormal results in the percentage of cells expressing the antigen or in its expression levels may be due to pathological conditions. It is advisable to be familiar with normal antigen expression patterns in order to interpret the results correctly<sup>4,5,6</sup>. The values obtained from healthy individuals may vary between laboratories. It is recommended that each laboratory establish its own normal ranges.

**CHARACTERISTICS**

SPECIFICITY

Blood samples were obtained from healthy Caucasian donors and treated with CD3 FITC (Clone: 33-2A3) and CD19 PE (Clone: A3-B1) monoclonal antibodies. Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Blood samples were processed using a direct fluorescence labelling method.

**CD3 FITC**

	Leukocytes		
	Lymphocytes	Monocytes	Granulocytes
SPN1	89.38	6.96	8.08
SPN2	82.87	3.33	8.93
SPN3	80.4	3.22	4.67
SPN4	84.98	1.38	3.48
SPN5	78.98	7.80	7.36
SPN6	71.39	6.00	5.68
SPN7	87.82	1.41	7.25
SPN8	79.52	3.69	4.11
SPN9	91.32	3.73	5.94
SPN10	62.53	4.78	3.22

Statistics	%Lymphocytes +	%Monocytes +	%Granulocytes +
N	10	10	10
Average	80.92	4.23	5.87
Median	81.635	3.71	5.81
Standard deviation	8.709092	2.162298	1.991240
Minimum	62.53	1.38	3.22
Maximum	91.32	7.80	8.93

**CD19 PE**

	Leukocytes		
	Lymphocytes	Monocytes	Granulocytes
SPN1	36	6.45	0.28
SPN2	2.26	18.10	0.34
SPN3	26.42	17.58	0.29
SPN4	4.47	9.70	0.58
SPN5	13.17	9.13	0.40
SPN6	9.39	5.27	0.29
SPN7	13.71	5.17	0.46
SPN8	11.24	3.13	0.27
SPN9	6.6	8.81	0.36
SPN10	11.08	4.79	0.53

Statistics	%Lymphocytes +	%Monocytes +	%Granulocytes +
N	10	10	10
Average	26.87	17.63	0.76
Median	11.16	7.63	0.35
Standard deviation	10.30981431	5.205606	0.110352969
Minimum	2.26	3.13	0.27
Maximum	26.42	18.10	0.58

SENSITIVITY

The sensitivity of CD3 FITC and CD19 PE monoclonal antibodies is determined by labelling a blood sample from a healthy donor. Increasing concentrations of each antibody (deliberate) are used to determine the consistency of the conjugated monoclonal antibody. This provides an indication of its reliability during normal use.

**CD3 FITC**

Model	R	R <sup>2</sup>	R <sup>2</sup> adjusted	Standard error of the estimate
1	.997 (a)	.993	.992	.63659

(a) Predictors: (Constant), Expected

**CD19 PE**

Model	R	R <sup>2</sup>	R <sup>2</sup> adjusted	Standard error of the estimate
1	.997 (a)	.994	.993	.17612

(a) Predictors: (Constant), Obtained

REPRODUCIBILITY

The reproducibility of the CD3 FITC and CD19 PE monoclonal antibodies was determined using 10 samples for each antibody in three ranges of values for CD3+ and CD19+, high, medium, and low. Thus, a total of 30 samples were labelled with CD3 and CD19. In this way, reproducibility was demonstrated across the entire range of values.

The 10 values in each range were labelled, processed and analysed separately. Lymphocytes were selected for analysis of the total percentage of cells for each of the three ranges.

To conduct this study, blood was obtained from normal donors expressing high levels of lymphocytes. The medium and low ranges were obtained by mixing known lymphocytes in appropriate ratios, while maintaining the total cell concentration for the three ranges.

The study was conducted in three independent laboratories, each of which separately obtained, labelled and analysed the blood samples.

## CD3 FITC

	HIGH %	MEDIUM %	LOW %
SAMPLE 1	29.48	23.06	14.44
SAMPLE 2	29.51	22.18	14.74
SAMPLE 3	29.51	25.54	15.99
SAMPLE 4	29.7	25.78	16.38
SAMPLE 5	29.34	25.97	15.58
SAMPLE 6	29.18	25.32	15.88
SAMPLE 7	28.12	24.98	16.53
SAMPLE 8	29.17	25.17	15.76
SAMPLE 9	28.87	25.26	16.24
SAMPLE 10	29.6	25.36	15.91

Statistics	HIGH %	AVERAGE %	LOW %
N	10	10	10
Average	29.241162	24.80296	15.71786494
CV (%)	1.59338133	4.973496	4.2996180
Standard deviation	0.465923	1.233574	0.67580815
Minimum	28.12	22.18	14.44
Maximum	29.7	25.97	16.53

## CD19 PE

	HIGH %	MEDIUM %	LOW %
SAMPLE 1	13.99	5.67	0.77
EXHIBITION 2	13.95	5.73	0.51
SAMPLE 3	14.4	5.4	0.61
SAMPLE 4	14.11	5.67	0.33
SAMPLE 5	14.14	5.7	0.48
SAMPLE 6	14.1	5.66	0.54
SAMPLE 7	14.19	5.61	0.53
SAMPLE 8	14.06	5.76	0.47
SAMPLE 9	14.14	5.6	0.49
SAMPLE 10	14	5.89	0.53

Statistics	HIGH %	MEDIUM %	LOW %
N	10	10	10
Average	14.10696	5.6664578	0.504887
CV (%)	0.90582	2.2205434	22.09888
Standard deviation	0.127784	0.125826159	0.111574589
Minimum	13.95	5.4	0.33
Maximum	14.4	5.89	0.77

## WARRANTY

Immunostep products are guaranteed in terms of quantity and content as indicated on the product label at the time of delivery to the customer. Immunostep disclaims any other warranty. Immunostep's liability is limited to the replacement of the products or a refund of the purchase price.

## REFERENCES

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