

# Anti-Human CD3 (33-2A3)

Fluorochrome	Reference	Test
FITC	3F1-100T	100 test
PE	3PE1-100T	100 test
APC	3A1-100T	100 test



## PRODUCT DESCRIPTION

**Other Names:** T3, CD3 $\epsilon$

**Description:** The anti-CD3 monoclonal antibody derives from the hybridisation of mouse SP2 myeloma cells and spleen cells from BALB/c mice immunised with human T lymphocytes. The antibody is formed by an IgG2a heavy chain and a kappa light chain.

**Clone:** 33-2A3

**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 CD3 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 ( $\mu$ g/ml)
FITC (Fluorescein isothiocyanate)	50 ug in 2 ml	25
PE (R-Phycoerythrin)	10 ug in 2 ml	5
APC (Allophycocyanin)	20 ug in 2 ml	10

## RECOMMENDED USAGE

Immunostep's CD3, clone 33-2A3, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample lymphocytes that express CD3 using flow cytometry.

## CLINICAL RELEVANCE

The Immunostep CD3 monoclonal antibody may also be used, in combination with other indicators, for the diagnosis or prognosis of some immunodeficiency diseases, including agammaglobulinemia and the severe combined immunodeficiency disease (SCID), which exhibit decreased percentages of T lymphocytes<sup>(1-6)</sup>.

Decreased percentages of T lymphocytes may also be observed in some autoimmune diseases, such as systemic lupus erythematosus (SLE), multiple sclerosis and Sjogren's disease, as well as in certain viral diseases caused by cytomegalovirus and Epstein-Barr virus. In general, diseases that have decreased cellular immunocompetence as a component may exhibit decreases in CD3+ mature T lymphocytes.

In addition, a large number of leukemias are T cell-related disorders, in which antibodies recognizing the CD3/TCR complex are an important diagnostic tool. Differentiation between AML, T-ALL and B-ALL can be made by immunophenotyping with (early) differentiation markers. Cell surface detection using CD3 antibodies can be applied for detection of mature T-ALL, while immature T-ALL express cytoplasmic (cyCD3) but no surface CD3.

CD3 antibodies are used, in the characterization of various subtypes of chronic lymphoid leukemias. Examples of these chronic T cell leukemias are T-CLL (Sezary Syndrome) and the peripheral T cell lymphoma (ATLL) which co-express CD3, CD2, CD4 and CD5 antigens. The NK cell lymphoma or the intestinal T cell lymphoma, co-express CD3, CD2 and CD8.

## PRINCIPLES OF THE TEST

The anti-CD3 monoclonal antibody binds to the surface of cells that express the CD3 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.
- 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)<sup>7-9</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-100
PE		ICIGG2APE-50
APC		ICIGG2AA-50

- 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 CD3 and determine the percentage of stained cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD3, so as to evaluate and correct the unspecific binding of lymphocytes (*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 peripheral blood stained:

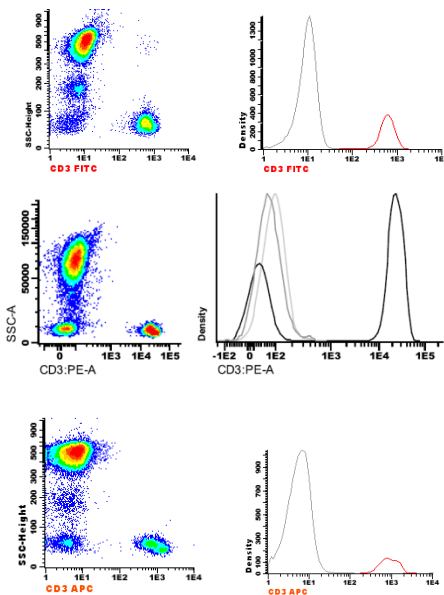


Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of the CD3+ lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

### 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.

- 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.
- 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<sup>10,11</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

The anti-CD3 antibody, clone 33-2A3, was included in the 2nd International Workshop on Human Leukocyte Differentiation Antigens (HLDA).

The antibody is directed against the CD3 antigen, also known as T3 or CD3ε, reacts with 85% of peripheral blood T lymphocytes, 70% of thymocytes, the majority of T cell chronic lymphocytic leukaemias, Sezary leukaemias and approximately 70% of acute lymphoblastic leukaemias of T cell origin.<sup>13</sup>

### LINEARITY

Linearity of the Immunostep CD3 monoclonal antibody was determined by staining Jurkat cell line as positive population and Ramos cell line as negative population. 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%.

Thereafter cells were incubated with the antibody according 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 shown in the following table:

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
CD3 FITC	0,99 <sup>a</sup>	0,99	0,99	0,63
CD3 PE	0,99 <sup>a</sup>	0,98	0,98	0,66
CD3 APC	0,99 <sup>a</sup>	0,98	0,98	0,77

a. Predictors: (Constant), % Expected

Data confirm the linearity and consistency of the conjugated monoclonal antibody as opposed to small variations (but deliberate). It provides an indication of its reliability during its normal use.

### INTRA-LABORATORY REPRODUCIBILITY

Reproducibility for the Immunostep CD3 monoclonal antibody was determined by performing 10 replicated determinations of anticoagulated blood samples from three healthy donors of three CD3+ ranges; high, medium and low. Thus, a total of 30 determinations were performed for each form of CD3 on the same day and using the same cytometer.

Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

The results obtained are summarized in the following table:

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	
CD3 APC	High	10	21,00	24,37	22,30	1,01
	Medium	10	6,14	6,63	6,39	0,16
	Low	10	1,16	1,47	1,33	0,10
	Valid N (listwise)	10				
CD3 PE	High	10	24,53	27,22	25,76	0,96
	Medium	10	21,70	23,47	22,69	0,44
	Low	10	20,12	21,75	20,98	0,52
	Valid N (listwise)	10				
CD3 FITC	High	10	28,12	29,70	29,24	0,46
	Medium	10	22,18	25,97	24,86	1,23
	Low	10	14,44	16,53	15,74	0,67
	Valid N (listwise)	10				

## 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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