# Anti-Human CD3 (33-2A3)

| | REF | Σ | | [A] | |
|---|---|---|---|---|---|
| PURE | 3PU1 | 1 mg | 10 µL/test | 1 mg/ml | |
| CF-Blue | 3CFB1-100T | 100 test | 5 µL/test | 0,05 mg/ml | |
| APC-C750 | 3AC750-100T | 100 test | 5 µL/test | 0,2 mg/ml | |
| APC | 3A1-X-100T | 100 test | 20 µL/test | 0,05 mg/ml | RUO |
| PerCP-Cyanine5.5 | 3PPC5.5-100T | 100 test | 5 µL/test | 0,2 mg/ml | |
| PerCP | 3PP1-100T | 100 test | 20 µL/test | 0,2 mg/ml | |
| PE | 3PE1-100T | 100 test | 20 µL/test | 0,05 mg/ml | |
| FITC | 3F1-100T | 100 test | 20 µL/test | 0,05 mg/ml | |

## 1. PRODUCT DESCRIPTION

**Clone:** 33-2A3;
**Isotype**: IgG2a;
**Tested application**: flow cytometry;
**Immunogen**: The anti-CD3 monoclonal antibody derives from human leukocytes;
**Species reactivity**: Human;
**Storage instruction:** store in the dark at 2-8 °C;
**Storage buffer:** aqueous buffered solution containing protein stabilizer and 0.09% sodium azide ($NaN_3$);
**Recommended usage**: Immunostep's CD3, clone 33-2A3, is a monoclonal antibody intended for the identification and enumeration of human T cells subsets using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for $10^6$ cells;
**Presentation**: liquid;
**Source**: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
**Purification**: Affinity chromatography;
**Other names**: Leu-4, T3;
**Gene ID:** 915;
**Molecular weight:** 22/26/30 kDa.

## 2. ANTIGEN DETAILS

**Large description:** The monoclonal antibody is directed against the CD3- antigen (T3-antigen), which is expressed on human T lymphocytes. The monoclonal antibody reacts with 80-90% human peripheral T lymphocytes and medullary thymocytes. The monoclonal antibody does not react with B-cells, monocytes, granulocytes and platelets. The monoclonal antibody is mitogenic for resting T lymphocytes and it blocks the cytolytic activity of CTL clones.[1-5]

## 3. 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.

## 4. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

## 5. PROTOCOL

**Direct Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

**Indirect Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

## 6. REFERENCES


1. Bohnhorst JO, Bjorgan MB, Thoen JE, Natvig JB, Thompson KM. Bm1-Bm5 classification of peripheral blood B cells reveals circulating germinal center founder cells in healthy individuals and disturbance in the B cell subpopulations in patients with primary Sjogren's syndrome. J Immunol2001 Oct 1;167(7):3610-8.
2. Buxade M, Ramirez-Alvarado M, Fernandez-Troy N, MacKenzie S, Casaroli-Marano RP, Vilella R, et al. Integrating signals from T-cell receptor and serum by T cells enhance translation of tumour necrosis factor-alpha. Immunology2001 Apr;102(4):416-25.
3. Gimferrer I, Calvo M, Mittelbrunn M, Farnos M, Sarrias MR, Enrich C, et al. Relevance of CD6-mediated interactions in T cell activation and proliferation. J Immunol2004 Aug 15;173(4):2262-70.
4. Gimferrer I, Farnos M, Calvo M, Mittelbrunn M, Enrich C, Sanchez-Madrid F, et al. The accessory molecules CD5 and CD6 associate on the membrane of lymphoid T cells. J Biol Chem2003 Mar 7;278(10):8564-71.
5. Kleijn M, Proud CG. The regulation of protein synthesis and translation factors by CD3 and CD28 in human primary T lymphocytes. BMC Biochem2002 May 17;3:11.


## 7. EXPLANATION OF SYMBOLS

| | |
|---|---|
| | Form |
| REF | Catalog reference |
| Σ | Contains sufficient for > test |
| | Regulatory Status |
| | Quantity per test |
| RUO | Research Use Only |
| [A] | Concentration |
| | Manufacturer |

## 8. MANUFACTURED BY:


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