Anti-Human CD19 (A3-B1)

	REF	Σ		[A]	
PURE	19PU1	1 mg	1 mg/ml	10 mg/ml	
PURE	19PU1-01MG	100 test	1 µg/test	10 mg/ml	RUO
PerCP-Cyanine5.5	19PPC5.51-100T	100 test	5 µL/test	0,2 mg/ml	
APC-C750	19AC750-100T	100 test	5 µL/test	0,2 mg/ml	

1. PRODUCT DESCRIPTION

Clone: A3-B1;
Isotype: IgG2a;
Tested application: flow cytometry;
Immunogen: The anti-CD19 monoclonal antibody derives from human tonsil;
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 CD19, clone A3-B1 is a monoclonal antibody intended for the identification and enumeration of human B cells 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: B-lymphocyte antigen CD19, B-lymphocyte surface antigen B4, Differentiation antigen CD19, T-cell surface antigen Leu-12, B4;
Gene ID: 930;
Molecular weight: Ig superfamily, type I transmembrane glycoprotein, 95 kDa.

2. ANTIGEN DETAILS

Large description: The monoclonal antibody is directed against the CD19- antigen (B4-antigen), which is expressed on human B lymphocytes. The monoclonal antibody is B lineage-specific and reacts with early B-cell precursors, pre-pre-B-cells, pre-B- cells, B-cells, intermediate B-cells, mature B-cells and some plasmacytoid cells, also reacts with pre-B-cell- lines, B lymphoblastoid cell-lines and Burkitt cell- lines, and with 50% of myeloma cell-lines. Plasma cells were found to be negative.[1-4]

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. Pezzutto A, Dorken B, Rabinovitch PS, Ledbetter JA, Moldenhauer G, Clark EA. CD19 monoclonal antibody HD37 inhibits anti-immunoglobulin-induced B cell activation and proliferation. J Immunol1987 May 1;138(9):2793-9.
2. Sato S, Ono N, Steeber DA, Pisetsky DS, Tedder TF. CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. J Immunol1996 Nov 15;157(10):4371-8.
3. Carter RH, Doody GM, Bolen JB, Fearon DT. Membrane IgM-induced tyrosine phosphorylation of CD19 requires a CD19 domain that mediatesassociation with components of the B cell antigen receptor complex. J Immunol1997 Apr 1;158(7):3062-9.
4. Braylan RC, Orfao A, Borowitz MJ, Davis BH. Optimal number of reagents required to evaluate hematolymphoid neoplasias: results of an international consensus meeting. Cytometry2001 Feb 15;46(1):23-7.

7. EXPLANATION OF SYMBOLS

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

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