Anti-Human CD21 (HI21a)

Form	REF	Test	Quantity per test		Regulatory status
CF-Blue	21CFB-100T	100 test	20 µL/test	2 mg/ml	RUO

1. PRODUCT DESCRIPTION

Clone: HI21a;
Isotype: IgG2a;
Tested application: flow cytometry;
Immunogen: The anti-CD21 monoclonal antibody derives from tonsil cells;
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 CD21, clone HI21a is a monoclonal antibody intended for the identification and enumeration of mature B cells, follicular dendritic cells and some epithelial 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: Complement receptor type 2 (Cr2), Complement C3d receptor, Epstein-Barr virus receptor (EBV receptor), C3DR;
Gene ID: 1380;
Molecular weight: 145 kDa.

2. ANTIGEN DETAILS

Large description: CD21 reacts with the C3d complement fragment and with Epstein Barr virus (EBV) receptors, found on mature B cells, follicular dendritic cells, andsome epithelial cells. It is also weakly expressed on a subset of mature T cells and thymocytes.

This clone also cross-reacts with a major subset of, but not all, peripheral blood CD20 + lymphocytes of baboon, and both rhesus and cynomolgus macaque monkeys. A subset of CD3 + cells is also CD21 +. The monoclonal antibody is directed against the CD21-antigen, which is expressed on normal Ig-positive B-cells from peripheral blood and lymphoid tissues and on dendritic cells of germinal centres. Its expression is lost on activated B-cells. The distinct distribution among B-cell malignancies differs from other B-cell markers e.g. CD19 and CD20.[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. Escribano L, Orfao A, Diaz-Agustin B, Villarrubia J, Cervero C, Lopez A, et al. Indolent systemic mast cell disease in adults: immunophenotypic characterization of bone marrow mast cells and its diagnostic implications. Blood1998 Apr 15;91(8):2731-6.
2. Escribano L, Orfao A, Villarrubia J, Diaz-Agustin B, Cervero C, Rios A, et al. Immunophenotypic characterization of human bone marrow mast cells. A flow cytometric study of normal and pathological bone marrow samples. Anal Cell Pathol1998;16(3):151-9.
3. 3. Reimann KA, Waite BC, Lee-Parritz DE, Lin W, Uchanska-Ziegler B, O'Connell MJ, et al. Use of human leukocyte-specific monoclonal antibodies for clinically immunophenotyping lymphocytes of rhesus monkeys. Cytometry1994 Sep 1;17(1):102-8.
4. Sopper S, Stahl-Hennig C, Demuth M, Johnston IC, Dorries R, ter Meulen V. Lymphocyte subsets and expression of differentiation markers in blood and lymphoid organs of rhesus monkeys. Cytometry1997 Dec 1;29(4):351-62.

7. EXPLANATION OF SYMBOLS

Symbol	Meaning
	Form
REF	Catalog reference
Σ	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

8. MANUFACTURED BY:

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