

# Anti-Human CD33 (HIM3-4)

					
APC	33A-100T	100 test	20 µL/test	0,05 mg/ml	

## 1. PRODUCT DESCRIPTION

**Clone:** HIM3-4;  
**Isotype:** IgG1;  
**Tested application:** flow cytometry;  
**Immunogen:** The anti-CD33 monoclonal antibody derives from KGla Cell Line;  
**Species reactivity:** Human, Cross-Reactivity: Chimpanzee;  
**Storage instruction:** store in the dark at 2-8 °C;  
**Storage buffer:** aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN<sub>3</sub>);  
**Recommended usage:** Immunostep's CD33, clone HIM3-4 is a monoclonal antibody intended for the identification and enumeration of Siglec-3 using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10<sup>6</sup> cells;  
**Presentation:** liquid;  
**Source:** Supernatant proceeding from an in vitro cell culture of a cell hybridoma;  
**Purification:** Affinity chromatography;  
**Other names:** Myeloid cell surface antigen CD33, Sialic acid-binding Ig-like lectin 3, Siglec-3, gp67, p67;  
**Gene ID:** 945;  
**Molecular weight:** 67 kDa.

## 2. ANTIGEN DETAILS

**Large description:** The monoclonal antibody is directed against the CD33-antigen, which is expressed on human myelomonocytic cells; monocytes, granulocytes (weakly), myeloid progenitors and mast cells. The monoclonal antibody reacts in the bone marrow from myeloblasts up to myelocytes. CD33-antigen is found on CFU-GEMM, CFU-GM, CFU-G, CFU-M and with erythroid CFU-E but not on earlier precursors. CD33 does not react with normal human peripheral B-cells, T-cells and platelets. CD33 reacts weakly with blast cells in 70% of patients with Acute Myeloid Leukaemia (AML) and in 30% of adult patients with Acute Lymphoblastic Leukaemia (ALL)<sup>(1-6)</sup>

## 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](http://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 acquire 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 acquire 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.

## • REFERENCES

1. Favaloro EJ, Bradstock KF, Kabral A, Grimsley P, Zowtyj H, Zola H. Further characterization of human myeloid antigens (gp160,95; gp150; gp67): investigation of epitopic heterogeneity and non-haemopoietic distribution using panels of monoclonal antibodies belonging to CD-11b, CD-13 and CD-33. Br J Haematol1988 Jun;69(2):163-71.
2. Favaloro EJ, Moraitis N, Koutts J, Exner T, Bradstock KF. Endothelial cells and normal circulating haemopoietic cells share a number of surface antigens. Thromb Haemost1989 Apr 25;61(2):217-24.
3. Freeman SD, Kelm S, Barber EK, Crocker PR. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. Blood1995 Apr 15;85(8):2005-12.
4. Nakamura Y, Noma M, Kidokoro M, Kobayashi N, Takei M, Kurashima S, et al. Expression of CD33 antigen on normal human activated T lymphocytes. Blood1994 Mar 1;83(5):1442-3.
5. Ulyanova T, Blasioli J, Woodford-Thomas TA, Thomas ML. The sialoadhesin CD33 is a myeloid-specific inhibitory receptor. Eur J Immunol1999 Nov;29(11):3440-9.
6. Knapp W. Leucocyte typing IV : white cell differentiation antigens. Oxford: Oxford University Press; 1989.

## 7. EXPLANATION OF SYMBOLS

	Form
	Catalog reference
	Contains sufficient for > test
	Quantity per test
	Regulatory Status
	Research Use Only
	Concentration
	Manufacturer

## 8. MANUFACTURED BY:



### IMMUNOSTEP S.L.

**Address:** Avda. Universidad de Coimbra, s/n  
Cancer Research Center (C.I.C)  
Campus de Unamuno  
37007 Salamanca (Spain)

**Telf./fax:** (+34) 923 294 827

**E-mail:** [info@immunostep.com](mailto:info@immunostep.com)  
[www.immunostep.com](http://www.immunostep.com)