Anti-Human CD33 (HIM3-4)

Fluorochrome	Reference	Test
FITC	33F-100T	100 test
PE	33PE-100T	100 test

IVD CE immunostep we know how

PRODUCT DESCRIPTION

Other Names: Myeloid cell surface antigen CD33, Sialic acid-binding Ig-like lectin 3, Siglec-3, gp67, p67
Description: The anti-CD33 monoclonal antibody derives from KG1a Cell Line.
Clone: HIM3-4
Isotype: Mouse IgG1, kappa
HLDA: Anti CD33 clone HIM3-4, was included in the fifth International Workshops on Human Leucocyte Differentiation Antigens, WS code MA112
Reactivity: Human
Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.
Purification: Affinity chromatography.
Composition: Mouse anti-human CD33 monoclonal antibody conjugated with a fluorochrome and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN_3).

Fluorochrome	Reagent provided	Concentration (µg/ml)
FITC (Fluorescein isothiocyanate)	200 ug in 2ml	100
PE (R-Phycoerythrin)	50 ug in 2 ml	25

RECOMMENDED USAGE

Immunostep's CD33, clone HIM3-4, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of human sample leucocytes that express CD33 using flow cytometry.

CLINICAL RELEVANCE

CD33 antigen is a useful marker for the diagnosis of non-lymphoid leukaemia cells. The CD33 antigen is earlier expressed in the cytoplasm (cyCD33) than the expression on the cell membrane (mCD33) of the myeloblasts. It is more valuable using cyCD33 and cyCD13 to diagnose leukaemia of AML-M0 as the CD13 antigen is also earlier expressed in the cytoplasm (cyCD13) than the expression on the cell membrane (mCD13) of the myeloblasts. [1-5]

PRINCIPLES OF THE TEST

The anti-CD33 monoclonal antibody binds to the surface of cells that express the CD33 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

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 ⚠

a) 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
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) 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)[6,7]. 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 IgG1	ICIGG1F-100UG
PE	Mouse IgG1	ICIGG1PE-50UG

- 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 10^6 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 CD33 and determine the percentage of stained cells.

It is necessary to use an isotype control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD33, so as to evaluate and correct the unspecific binding of leucocytes *(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 stained cells:

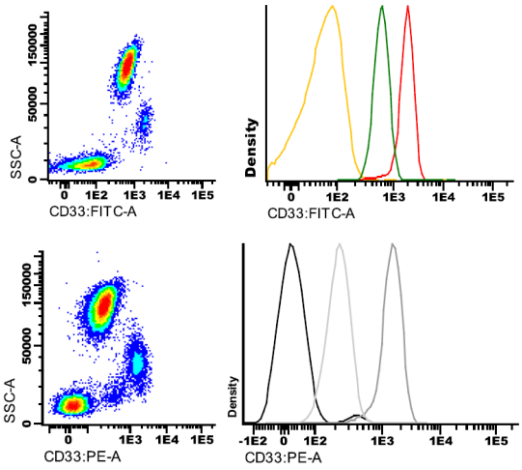

Fig. 1 On the left, a biparametric diagram of the average fluorescence intensity of the CD33+ lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

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.
5. 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
6. kept at room temperature immediately prior to incubation with the monoclonal antibody.
7. 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 result[8,9,10]

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

Anti CD33 clone HIM3-4, was included in the fifth International Workshops on Human Leucocyte Differentiation Antigens, WS code MA112.

CD33 is expressed on myelomonocytic cells cells. To evaluate the reagent's specificity (cross-reactivity with other cell populations), 10 blood samples from healthy donors were studied, stained with an adequate isotype control and the MAb to study.

Blood samples obtained from Caucasian healthy normal donors were stained with Immunostep CD33 monoclonal antibody. Non-specific fluorescence identified by the conjugated isotype control was analysed. Cells contained in the lymphocyte, monocyte, granulocyte, platelets and erythrocytes regions were selected for analysis. Blood samples were processed by a Staining Cell Surface Antigens for Flow Cytometry Protocol.

The results obtained are shown in the following table:

Descriptive Statistics					
FITC					
	N	Minimum	Maximum	Mean	Std. Deviation
% Isotype control	10	,01	,91	,2000	,31045
% T lymphocytes	10	,00	,04	,0070	,01252
% B lymphocytes	10	,00	,03	,0070	,00949
% Granulocytes	10	,01	1,28	,2420	,40458
% Platelets	10	,03	,26	,1130	,07273
% Erythrocytes	10	,02	,10	,0560	,02797
Valid N (listwise)	10				
PE					
% Isotype control	10	,00	,53	,1840	,19699
% Platelets	10	,04	,45	,2380	,14711
% T Lymphocyte	10	,00	,12	,0360	,04551
% Erythrocytes	10	,01	,46	,1660	,14645
% B Lymphocyte	10	,00	,03	,0070	,00949
Valid N (listwise)	10				

SENSIBILITY

Sensitivity of the Immunostep CD33 monoclonal antibody was determined by staining a positive cell line (U937) and a negative cell line (Jurkat). Cells were mixed in different proportions with a constant final number of $5X10^6$ cells to achieve different cell ratios from 0% positive cells to 100%.

Thereafter cells were incubated with the antibody according to the recommended amount and incubated 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.

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

	Model Summary[b]				
	R	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
FITC	,998[a]	,0996	,996	2,36546	y = 1.029x – 0,190
PE	,996[a]	,993	,992	3,09025	y = 1,000x + 1,965

a. Predictors: (Constant), % Expected

b. Dependent Variable: % Obtained

REPRODUCIBILITY

Reproducibility for the Immunostep CD33 conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each sample in each of three leukocyte ranges: high, medium and low. Three samples of each range were used. Thus, a total of 30 determinations were performed for each type of range. Thereby reproducibility was demonstrated throughout the entire measuring range.

The 30 determinations for each range were performed by the staining, processing and analysis of 3 separate samples. Monocytes CD33+ were selected for the analysis of percentage cells stained in each measure.

To perform this study, anti-coagulated blood was obtained from normal donors expressing a different percentage of leukocytes.

Descriptive Statistics

FITC					
Range	N	Minimum	Maximum	Mean	Std. Deviation
High	10	8,67	9,81	9,059	0,3822
Medium	10	5,48	6,13	5,757	0,2032
Low	10	4,93	5,55	5,256	0,21732
PE					
High	10	6,18	6,883	6,529	0,208
Medium	10	5,086	5,77	5,423	0,221
Low	10	6,18	6,883	6,529	0,208

ACCURACY or REPEATABILITY

To determine the repeatability of staining with this product, 10 different samples were stained with 3 different lots of this reagent. For each sample three different values were obtained of the mean fluorescence intensity (MFI) and the percentage of positive cells. The standard deviation mean and the IMF mean of ten results obtained were calculated. The results of the analysis are shown in the following chart:

	Average Mean	Average Std. Deviation	Pooled %CV
FITC			
% positive	5,5869	0,3349	5,99
IMF	71,029	5,5869	7,86
PE			
% positive	5,1625	0,1591	3,0005
IMF	2896,7500	220,2638	7,2681
Valid N (listwise)	30	30	30

As shown in the table, the results show excellent repeatability from lot to lot, both average %CV percentages of positive cells and MFI as show values.

*Note: Data analyzed with SPSS for Windows 11.0.1

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.

REFERENCES

1. Ana B Pérez-Oliva, María Martínez-Esparza, José J Vicente-Fernández, Rubén Corral-San Miguel, Pilar García-Peñarrubia1, and Trinidad Hernández-Caselles1,. Epitope mapping, expression and post-translational modifications of two isoforms of CD33 (CD33M and CD33m) on lymphoid and myeloid human cell. Glycobiology (2011) 21 (6): 757-770.

2. Melissa G Lechner, Carolina Megiel, Sarah M Russell, Brigid Bingham, Nicholas Arger, Tammy Woo and Alan L Epstein. Functional characterization of human Cd33+ And Cd11b+ myeloid-derived suppressor cell subsets induced from peripheral blood mononuclear cells co-cultured with a diverse set of human tumor cell lines. Journal of Translational Medicine 2011, 9:90

3. B Brichard, I Varis, D Latinne, V Deneys, M de Bruyere, P Leveugle and G Cornu. Intracellular cytokine profile of cord and adult blood monocytes. Bone Marrow Transplantation (2001) 27, 1081-1086.

4. Dzung H. Nguyen, Nancy Hurtado-Ziola, Pascal Gagneux, and Ajit Varki. Loss of Siglec expression on T lymphocytes during human evolution. PNAS, May 16, 2006, vol. 103, no. 20, 7765–7770

5. Dennis Sgroi, Aaron Nocks and Ivan Stamenkovic. A Single N-linked Glycosylation Site Is Implicated in the Regulation of Ligand Recognition by the I-type Lectins CD22 and CD33. August 2, 1996 The Journal of Biological Chemistry, 271, 18803-18809.

6. Procedures for the collection of diagnostic blood specimens by venipuncture- approved standard; Fifthedition (2003). Wayne PA: National Committee for Clinical Laboratory Standards; Document H3-A5.

7. Standard Procedures for the Collection of Diagnostic Blood Specimens", publicado por el National Committee for Clinical Laboratory Standards (NCCLS)

8. Quality assurance and immunophenotyping of lymphocytes; approved guideline (1998). Wayne PA: National Committee for Clinical Laboratory Standards; Document H42-A.

9. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. Am J Clin Pathol 100:111-5 (1993)

10. Reichert et al. Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 60:190-208 (1991)

MANUFACTURED BY

Immunostep S.L
Avda. Universidad de Coimbra, s/n
Cancer Research Center (CIC)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. (+34) 923 294 827
www.immunostep.com