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Product: FITC Anti-Human CD33
Other Names: Myeloid cell surface antigen CD33, Sialic acid-binding Ig-like lectin 3, Siglec-3, gp67, p67
Cat. Ref: 33F-100T
Reagent provided: 100 test (20µl/test)
Description: CD33 FITC is recommended for use in flow cytometry for identification of CD33m isoform[1] which is expressed on myeloid progenitors, monocytes, granulocytes, dendritic cells and mast cells. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide
Clone: HIM3-4
HLDA: Anti CD33 clone HIM3-4, was included in the fifth International Workshops on Human Leucocyte Differentiation Antigens, WS code MA112
Isotype: IgG1
Reactivity: Human.
Fluorochrome: Fluorescein isothiocyanate, FITC (Ex-Max: 494 nm/Em-Max: 520 nm). Recommended 488 nm ion argon laser, 502 LP filter and 530/30 detector-equipped flow cytometer.)

Storage: Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com).

Application: It is recommended for use in flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 20 µl/10^6 cells.

INTENDED PURPOSE.

CD33 FITC is a monoclonal antibody conjugated that may be used to identification of myeloid progenitors, monocytes, granulocytes, dendritic cells and mast cells.

TECHNICAL SUMMARY.

Reactivity: The monoclonal antibody is directed against the CD33-antigen (belonging to the Ig-supergene family), 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. The monoclonal antibody does not react with normal human peripheral B-cells, T-cells and platelets.

According to the last publications, the expression of the isoform CD33m, a CD33 transmembrane splice variant lacking the ligand-binding V immunoglobulin (Ig)-like domain, was detected by CD33 clone HIM3-4 and not by other clones[1].

Specificity: The antigen is a single chain transmembrane glycoprotein of molecular mass 67 kDa (364 AA.) expressed on monocytes, myeloid progenitor cells and myeloid leukaemias. The antigen has homology with CD22 and myelin associated glycoprotein. CD33 appears very early on myelomonocytic precursors and is expressed in both the myeloid and monocytic cell lineages. CD33 is absent from pluripotent stem cells but appears on myelomonocytic precursors after CD34. It then continues to be expressed on both the myeloid and monocyte lineages. The CD33 antigen functions as an adhesion molecule.

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.

The monoclonal antibody 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).

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PRINCIPLES OF THE TEST.

Immunostep CD33 FITC monoclonal antibodies bind to the surface and cytoplasm of cells that express the CD33 antigen. To identify these cells, peripheral blood leucocytes are incubated with the antibodies. Analysed by flow cytometry with an Helio-Neon laser at 488 nm.

REAGENTS.

Cluster Designation:	Anti-Human CD33
Clone:	HIM3-4
Isotype:	IgG1
Species:	Mouse
Composition:	IgG1 heavy chain Kappa light chain
Source:	Hybridome Cells
Method of Purification:	Affinity chromatography
Fluorochrome:	FITC Excitation wavelength 488 nm Emission wavelength 520 nm
Molar composition:	FITC/protein ±6.0 (3-8)
Reagents contents:	2 ml vial containing monoclonal antibody for 100 tests, containing protein stabilizer and ⩽0.09% sodium Azide, 0.02 M sodium phosphate, 0.15 M sodium chloride, pH 7.2
Reagent preparation:	Ready to use.

1. STATEMENTS, SETTINGS AND WARNINGS.

- Reagents contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
- Light exposure should be avoided. Use dim light during handling, incubation with cells and prior to analysis.
- Do not pipet by mouth.
- Samples should be handled as if capable of transmitting infection. Appropriate disposal methods should be used.
- The sample preparation procedure employs a fixative (formaldehyde). Contact is to be avoided with skin or mucous membranes.
- Do not use antibodies beyond the stated expiration dates of the products.
- Deviations from the recommended procedure enclosed within this product insert may invalidate the results of testing.
- FOR *IN VITRO* DIAGNOSTIC USE
- For professional use only.

2. APPROPIATE STORAGE CONDITIONS.

a. FITC
Keep in dark place at 2-8°C. DO NOT FREEZE. Avoid overexposure to light at room temperature.

3. EVIDENCE OF DETERIORATION.

Reagents should not be used if any evidence of deterioration or substantial loss of reactivity is observed. For more information, please contact with our technical service: tech@immunostep.com

- The normal appearance of the FITC conjugated monoclonal antibody is a clear, yellow liquid without aggregates or precipitates.

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4. SPECIMEN COLLECTION.

Collect venous blood samples into blood collection tubes using an appropriate anticoagulant (EDTA or heparin). For optimal results the sample should be processed within 6 hours of venepuncture. EDTA, ACD or heparin may be used if the blood sample is processed for analysis within 30 hours of venepuncture. ACD or heparin, but not EDTA, may be used if the sample is not processed within 30 hours of venepuncture. Samples that cannot be processed within 48 hours should be discarded.

If venous blood samples are collected into ACD for flow cytometric analysis, a separate venous blood sample should be collected into EDTA if a CBC is required.

Unstained anticoagulated blood should be retained at 20- 25°C prior to sample processing. Blood samples that are haemolysed, clotted or appear to be lipemic, discoloured or to contain interfering substances should be discarded.

Refer to "*Standard Procedures for the Collection of Diagnostic Blood Specimens*" published by the National Committee for Clinical Laboratory Standards (NCCLS) for additional information on the collection of blood specimens.

5. SAMPLE PREPARATION.

Staining Cell Surface Antigens for Flow Cytometry Protocol

1. Add 20 µL of CD33 FITC to a 12 x 75 mm polystyrene test tube. The 20 µL is a guideline only; the optimal volume should be determined by the individual laboratory
2. Transfer 100 µL of anticoagulated (EDTA) blood (10^6 cells) and mix gently with a vortex mixer.
3. The recommended negative control is a non-reactive FITC-conjugated antibody of the same isotype.
4. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.
5. Add Lysing Solution according to the manufacturer's directions to each sample and mix gently with a vortex mixer.
6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.
7. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
8. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.

Intracellular staining protocol

1. For each sample, add an appropriate volume of conjugated antibody directed to the cell surface antigen of interest and the appropriate isotype control. Incubate for 15 minutes in the dark at room temperature. (This step is only necessary is you want to perform a direct immunofluorescence staining for a cell surface antigen)
2. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
3. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid
4. Add 100 µl of IntraCell Reagent A, (Fixative), to each tube. Mix gently. Incubate in the dark at room temperature (20-25 °C) for 15 minutes.
5. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
7. Add 100 µl of IntraCell Reagent B (Permeabilization), to each tube. Add the appropriate volume of CD33 FITC for the intracellular antigen and the appropriate isotype control.
8. Incubate for 15 minutes in the dark at room temperature.
9. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.

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10. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
11. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: **www.immunostep.com**

6. MATERIALS REQUIRED BUT NOT SUPPLIED.

Isotype control reagents:	Mouse IgG1: FITC
Leucocyte gating reagent:	Mouse anti-human CD45: PerCP
Serofuge or equivalent centrifuge	
12 x 75 mm polypropylene centrifuge tubes	
Micropipette capable of dispensing 5 µl, 20 µl, 100 µl, and 500 µl volumes	
Blood collection tubes with anticoagulant	
Phosphate buffered saline (PBS)	
Trypan Blue or propidium iodide, 0.25% (w/v) in PBS for the determination of cell viability	
Lysing Solution	
Fixing Solution	
Flow cytometer:	Becton Dickinson FACSCaliburTM, Coulter Profile or equivalent 488 nm ion argon laser, 502 LP filter and 530/30 detector-equipped flow cytometer.

7. INTERPRETATION OF RESULTS.

a. *FLOW CYTOMETRY*

Analyze antibody-stained cells on an appropriate flow cytometer analyser according to the manufacturer instructions. The right angle light scatter or other scatter (SSC) versus forward angle light scatter (FSC) is collected to reveal the lymphocyte cell cluster. A gate is drawn for the Leukocytes. The fluorescence attributable to the FITC- conjugated monoclonal antibody is collected, and the percentage of antibody-stained is determined. An appropriate FITC-conjugated isotypic control of the same heavy chain immunoglobulin class and antibody concentration must be used to estimate and correct for non-specific binding. An analysis region is set to exclude background fluorescence and to include positively stained cells. The following histograms are representative of cells stained and region from a normal donor.

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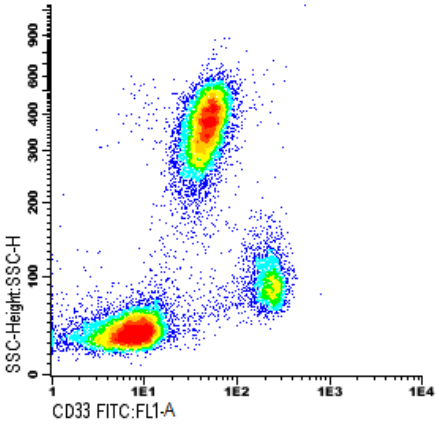

The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on leukocytes. Human peripheral blood were stained with CD33 FITC and CD45 PerCP. Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

8. QUALITY CONTROL PROCEDURES.

Non-specific fluorescence identified by the FITC conjugated isotypic control is usually less than 2% in normal individuals. Non-specific fluorescence identified by the PE and APC conjugated isotypic controls are usually less than 4% in normal individuals. If the background level exceeds these values, test results may be in error. Increased non-specific fluorescence may be seen in some disease states.

A blood sample from each normal and abnormal donor should be stained with the CD45 Pan-leukocyte and CD14 Pan-monocyte monoclonal antibodies. When used in combination, these reagents assist in identifying the cells analysis region, and distinguish lymphocytes from monocytes, granulocytes and unlysed or nucleated red cells and cellular debris.

A blood sample from a healthy normal donor should be analyzed as a positive control on a daily basis or as frequently as needed to ensure proper laboratory working conditions. Each laboratory should establish their own normal ranges, since values obtained from normal samples may vary from laboratory to laboratory.

An appropriate isotype control should be used as a negative control with each patient sample to identify non-specific Fc binding to lymphocytes. An analysis region should be set to exclude the non-specific fluorescence identified by the isotypic control, and to include the brighter fluorescence of the lymphocyte population that is identified by the specific antibody.

Refer to the appropriate flow cytometer instrument manuals and other available references for recommended instrument calibration procedures.

9. LIMITATIONS OF THE PROCEDURE.

1. Incubation of antibody with cells for other than the recommended time and temperature may result in capping or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.
3. Abnormal cells or cell lines may have a higher antigen density than normal cells. This could, in some cases, require the use of a larger quantity of monoclonal antibody than is indicated in the procedures for Sample Preparation.
4. Blood samples from abnormal donors may not always show abnormal values for the percentage of lymphocytes stained with a given monoclonal antibody. Results obtained by flow cytometric analysis should be considered in combination with results from other diagnostic procedures.
5. When using the whole blood method, red blood cells found in some abnormal donors, as well as nucleated red cells found in normal and abnormal donors may be resistant to lysis by lysing solutions. Longer red cell lysis periods may be needed to avoid the inclusion of unlysed red cells in the lymphocyte gated region.
6. Blood samples should not be refrigerated or retained at ambient temperature for an extensive period (longer than 24-30 hours) prior to incubating with monoclonal antibodies.
7. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the laser, as well as proper gate settings.

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8. Due to an unacceptable variance among the different laboratory methods for determining absolute lymphocyte counts, an assessment of the accuracy of the method used is necessary.
9. AI results need to be interpreted in the context of clinical features, complete immunophenotype and cell morphology, taking due account of samples containing a mixture of normal and neoplastic cells.

10. REFERENCE VALUES.

The cellular elements of human Bone Marrow include lymphocytes, monocytes, granulocytes, red blood cells and platelets.

Nucleated cells Percentage in the Bone Marrow

Cell type	Percentage
Progranulocytes	56,7
Neutrophils	53,6
Myeloblasts	0,9
Promyeloblasts	3,3
Promyelocytes	12,7
Metamyelocytes	15,9
Eosinophils	3,1
Basophils	<0,1
Proerythrocyte	25,6
Proerythrblasts	0,6
Basophil Erythroblast	1,4
Polycromatic Erythroblast	21,6
Ortocromatic Erythroblast	2
Megakaryocytes	<0,2
Lymphocytes	16,2
Plasma cells	2,3
Reticular cells	0,4

Normal human peripheral blood lymphocytes 20-47% (n=150% confidence interval)

Nucleated cells Percentage in Peripheral Blood of a Normal Patient

Cell type	Percentage	Number of event.
Red Blood Count		3,8 - 5,6 $X10^6$/µL
Platelets		150 - 450 $X10^3$/µL
White Blood Count (WBC)		4.3 - 10.0 $X10^3$/µL
Neutrophils	57 – 67 %	1,5 - 7.0 $X10^3$/µL
Lymphocytes*	25 – 33 %	1.0 - 4.8 $X10^3$/µL
T cell	56 – 82 % of lymphocytes	
T cell CD4+	60 % of T cells	
T cell CD8+	40 % of T cells	
Cell NK+	6 – 33 of lymphocytes	
B cell	7.7 – 22 of lymphocytes	
Monocytes	3 – 7 %	0.28 - 0.8 $X10^3$/µL
Eosinophils	1 – 3 %	0.05 – 0,25 $X10^3$/µL
Basophils	0 – 0,075 %	0,015 – 0,05 $X10^3$/µL
Reticulocyte	0,5 – 1,5 % of total Red Blood Cell	

Expected values for pediatrics and adolescents have not been established.

The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

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11. PERFORMANCE CHARACTERISTICS.

a. *SPECIFICITY*

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

Blood samples obtained from healthy normal donors of Caucasian were stained with Immunostep CD33 FITC monoclonal antibody. Non-specific fluorescence identified by the FITC conjugated isotypic 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

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
% Isotype control	10	,01	,91	,2000	,31045	,096
% T lymphocytes	10	,00	,04	,0070	,01252	,000
% B lymphocytes	10	,00	,03	,0070	,00949	,000
% Granulocytes	10	,01	1,28	,2420	,40458	,164
% Platelets	10	,03	,26	,1130	,07273	,005
% Erythrocytes	10	,02	,10	,0560	,02797	,001
Valid N (listwise)	10					

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.

b. *SENSIBILITY or LINEARITY*

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
,998[a]	,0996	,996	2,36546	y = 1.029x – 0,190

a. Predictors: (Constant), % Expected

b. Dependent Variable: % Obtained

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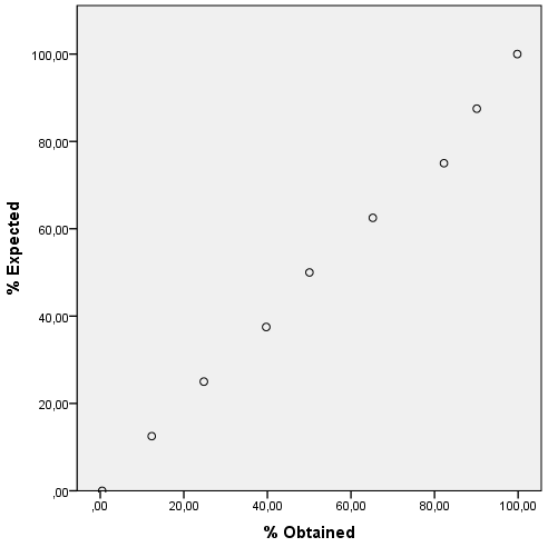

c. *REPRODUCIBILITY*

Reproducibility for the Immunostep CD33 FITC-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.

Low percentage of Leukocytes

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Sample 1	10	6,45	7,22	6,7330	,07082	,22396
Sample 2	10	8,62	9,27	9,0190	,06310	,19952
Sample 3	10	3,47	4,16	3,8350	,06435	,20348
Valid N (listwise)	10					

Normal percentage of Leukocytes

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Sample 1	10	5,12	5,91	5,4700	,08215	,25979
Sample 2	10	5,76	6,41	6,1480	,06385	,20192
Sample 3	10	4,38	4,99	4,6530	,06391	,20210
Valid N (listwise)	10					

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High percentage of Leukocytes

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Sample 1	10	6,45	7,22	6,7330	,07082	,22396
Sample 2	10	8,62	9,27	9,0190	,06310	,19952
Sample 3	10	3,47	4,16	3,8350	,06435	,20348
Valid N (listwise)	10					

**Note:* Data analyzed with SPSS for Windows 21*

d. 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
% positive	5,5869	0,3349	5,99
IMF	71,029	5,5869	7,86
Valid N (listwise)	30	30	

**Note:* Data analyzed with SPSS for Windows 21*

12. BIBLIOGRAPHY.

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