Anti-Human CD34 (581)

Fluorochrome	Reference	Test
PE	34PE-100T	100 test
APC	34A-100T	100 test

PRODUCT DESCRIPTION

Other Names: Hematopoietic progenitor cell antigen CD34
Description: The anti-CD34 monoclonal antibody derives from human CD34 cells.
Clone: 581
Isotype: Mouse IgG1, kappa
HLDA: V, WS Code MA27
Reactivity: Human
Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.
Purification: Affinity chromatography.
Compositión: Mouse anti-human CD34 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)
PE (R-Phycoerythrin)	25 ug in 2 ml	12,5
APC (Allophycocyanin)	30 ug in 2 ml	15

RECOMMENDED USAGE

Immunostep's CD34, clone 581, is a monoclonal antibody intended for *in vitro* diagnostic use in the identification and enumeration of early lymphohematopoietic stem and progenitor cells using flow cytometry.

CLINICAL RELEVANCE

CD34 antigen density is highest on early hematopoietic progenitor cells and decreases as cells mature. The antigen is absent on fully differentiated hematopoietic cells[1-5]

PRINCIPLES OF THE TEST

The anti-CD34 monoclonal antibody binds to the surface of cells that express the CD34 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
PE	Mouse IgG1	ICIGG1PE-50
APC		ICIGG1A-50

- 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 CD34 and determine the percentage of stainend cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration as that of the CD34, so as to evaluate and correct the unspecific binding of lymphocytes *(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 peripheral blood stained:

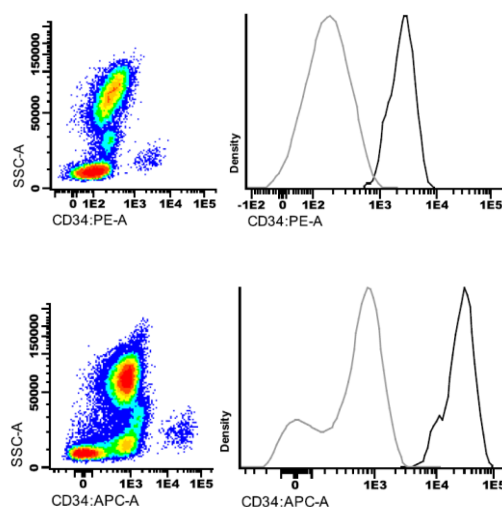

Fig. 1: On the left, a biparametric diagram of the average fluorescence intensity of the CD34+ lymphocyte population and its internal complexity (SSC) in a peripheral blood specimen from a healthy donor. On the right, a diagram of the same specimen in histogram format.

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 kept at room temperature immediately prior to incubation with the monoclonal antibody.
6. 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 results[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 CD34 clone 581, was included in the fifth International Workshops on Human Leucocyte Differentiation Antigens, WS code MA27.

The CD34 antigen is present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitors. The CD34 antigen is present on early myeloid cells that express the CD33 antigen but lack the CD14 and CD15 antigens and on early erythroid cells that express the CD71 antigen and dimly express the CD45 antigen. The CD34 antigen is also found on capillary endothelial cells and approximately 1% of human thymocytes. Normal peripheral blood lymphocytes, monocytes, granulocytes, and platelets do not express the CD34 antigen.

SENSIBILITY

Sensitivity of the Immunostep CD34 monoclonal antibody was determined by staining as positive an activated human CD34 transfected 293T cells and as negative the same line without activation. Cells were mixed in different proportions with a constant final number of 1 x 106 cells to achieve different cell ratios from 0% positive cells to 100%.

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

The results show an excellent correlation between the results obtained and expected based on the dilution used. CD34 sensibility was demonstrated from 1 x 105 to 1 x 106 cells in 1 x 106 total cells.

Model Summary

Model	R Square	Adjusted R Square	Std. Error of the Estimate	Linear regression
PE	,985	,983	4,52684	y = 0,897x – 1,697
APC	,985	,983	4,52684	y = 0,897x – 1,697

a. Predictors: (Constant), % Expected

REPRODUCIBILITY

Reproducibility for the Immunostep CD34 conjugated monoclonal antibodies was determined by performing 10 replicated determinations of each antibody in each of three ranges of lymphocytes; high, medium and low.
Thus, a total of 30 determinations were performed. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes CD19+ were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from three different donors expressing a high, medium and low percentage of Lymphocytes.

Descriptive Statistics

Percentage		N	Minimum	Maximum	Mean	Std. Deviation
High	Result	10	76,2	79,23	77,805	0,95003
	Valid N (listwise)	10				
Medium	Result	10	51,69	60,26	56,745	2,74539
	Valid N (listwise)	10				
Low	Result	10	3,95	15,98	8,361	3,31537
	Valid N (listwise)	10				

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.

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