Stem Cell Kit

Reference	Test
SCK	50 test
SCK2	50 test

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INTRODUCTION

Stem Cell Kit is a kit designed for quantitative determination of hematopoietic stem cells (HSCs) CD34 positive due to the specificity of the following antibodies:

The anti-CD45 antibody recognizes type C tyrosine receptor protein phosphatase, a protein of 220 kD member of Leukocyte Antigen family.

The anti-CD34 recognizes the progenitor hematopoietic cell protein antigen CD34, a monomeric type I membrane glycoprotein 110 kD present[13,14] in immature hematopoietic precursor cells from the bone marrow[17] and blood, and in endothelium capillary blood[15,16] from many tissues.

MATERIALS PROVIDED

The SCK reference includes a vial of CD45/CD34-positive cells, a CD45/ IgG1 isotype control vial, counting tubes, a vial of 7-AAD and a bottle of lysis solution 10X.

- Vial A: 1 ml at 20 µl/test. CD45 and CD34 are murine monoclonal antibodies. CD45 is clone HI30 and CD34 is clone 581. The reagent is in an aqueous solution and contains a stabilizing protein and 0.09% sodium azide (NaN3).
- Vial B: 1 ml at 20 µl/test. CD45 and mouse IgG1 isotype control are murine monoclonal antibodies. CD45 is clone HI30 and Mouse IgG1 isotype control is clone B11/6. The reagent is in an aqueous solution and contains a stabilizing protein and 0.09% sodium azide (NaN3).
- StepCount counting tubes (100): tubes for use in flow cytometry containing a known number of microspheres of 4.2 micron of diameter capable of emitting fluorescence (the number appears on the tube label)
- 7-AAD: It comes in 5 µl vials, with 500 µl required for 100 tests. 7-Amino-Actinomycin liquid and 0.09% sodium azide (NaN_3) to identify non viable cells
- 10X lysis solution (10 ML): consisting of ammonium chloride (NH_4Cl) to lyse erythrocytes and 0.09% sodium azide (NaN_3).

The SCK2 reference includes a vial of CD45 / CD34, counting tubes, a vial of 7-AAD and a bottle of lysis solution 10X.

- Vial A: 1 ml at 20 µl/test. CD45/CD34, Murine Monoclonal Antibodies CD45 is clone HI30 and CD34 is clone 581. The reagent is in an aqueous solution and contains a stabilizing protein and 0.09% sodium azide (NaN3).
- StepCount counting tubes (50): tubes for use in flow cytometry containing a known number of microspheres of 4.2 micron of diameter capable of emitting fluorescence (the number appears on the tube label)
- 7-AAD: It comes in 5 µl vials, with 250 µl required for 50 tests. 7-Amino-Actinomycin liquid and 0.09% sodium azide (NaN_3) to identify non-viable cells
- 10X lysis solution (10 ML): consisting of ammonium chloride (NH_4Cl) to lyse erythrocytes and 0.09% sodium azide (NaN_3).

MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge
- 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
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

RECOMMENDED USAGE

The SCK (Stem Cell Kit) from Immunostep is designed for use in flow cytometry to determine the percentage and absolute counts of CD34+/CD45+ cells and viable cells, discriminating between non-viable cells. Additionally (only in the SCK reference), the number of false positives caused by non-specific staining can be detected with the use of a CD34/Isotype control.

With this kit, you can analyze any sample, including blood, bone marrow, mobilized blood, leukapheresis products, and umbilical cord blood.

CLINICAL RELEVANCE

The enumeration of CD34+ cells in the CMH plays a crucial role in both autologous and allogeneic transplantation. These cells are extensively utilized in the treatment of hematological malignancies, solid tumors, and autoimmune disorders. The success of rapid hematopoietic reconstitution is largely dependent on the reinfusion of a substantial number of CD34+ cells.

PRINCIPLES OF THE TEST

The kit enables the identification of CD34+ cells and correlates their quantity with a known number of spheres. This process discriminates between viable and non-viable cells while eliminating false positives resulting from non-specific staining of the isotype control.

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)[2,3]. 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.

SAMPLE PREPARATION

1. It is recommended to make a total cell count before proceeding to trial. If the number of cells is greater than 50 x 10^3 cells / ul you should make a dilution with PBS / BSA (see Materials required but not provided) so that cell concentration decreases (remember scoring dilution).

2. Label two StepCount tubes as described below:
   - Tube N° 1: CD45/CD34/7-AAD
   - Tube N° 2 (only SCK): CD45/Isotype Control/7-ADD

3. Add 20 µl of A reagent: CD45 FITC / CD34 PE in tube N°1. Pipette 20 µl of B reagent: CD45 FITC / isotype control PE in tube N°2 (only SCK). Do not touch the sediment area.

4. Add 5 µl of 7-AAD in each tube.

5. Add the sample in each tube and mix well on vortex. The recommended volume is between 20 and 100 ul (note the volume).

6. Incubate in the dark at room temperature (20-25° C) for 15 minutes or at 4° C for 30 minutes.

7. Add 0,45 ml of 1X de Ammonium chloride lysis solution in each tuve. Mix well on vortex and incubate in the dark for 10 minutes or until the sample is lised.

8. Acquire in a flow cytometry or keep at 2-8° C in the dark until the analysis. Samples should be acquired within the 1 hour after lysis.

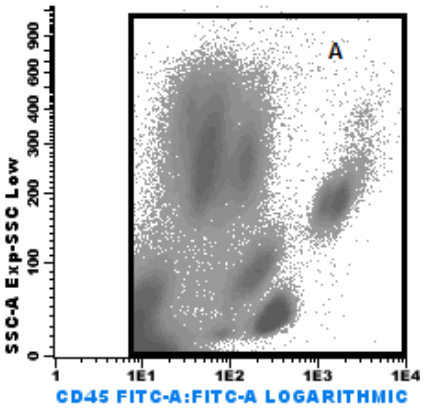
FLOW CYTOMETRY ANALYSIS

Before acquiring samples, verify that the cytometer is correctly aligned and standardized for light scatter. Fluorescence compensation is established following the manufacturer's cytometer instructions.
FSC and SSC parameters must be set in the linear amplification while the fluorescence intensity (FL1 parameters, FL2, FL3 FL4 ...) should be adjusted in the logarithmic amplification.

Before acquiring samples, adjust at minimum the threshold or discriminator in the FSC parameter to minimize waste and ensure that the population of interest will be included in the analysis.

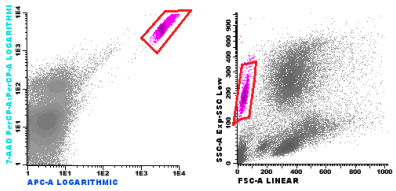
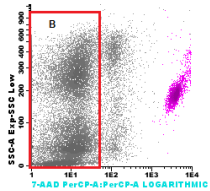
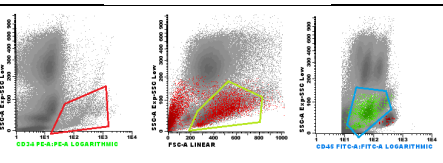
Before purchasing the flow cytometer, it is recommended to shake the samples manually and gently to ensure complete resuspension of cells and microspheres.

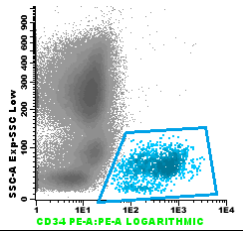
Select region A as shown in the picture for store the events in this region during acquisition in the cytometry:



Acquire and analyze sample in the tube as much as possible. It is recommended to acquire at low or medium speed to avoid the formation of cell aggregates. If you will not acquire the entire sample is advisable to stop every 4 minutes, shake the tube manually and proceed with the acquisition.

Example of analysis:

STAGE	IMAGE
Stage 1: selection of microspheres population	
Stage 2: selection of viable cells population (section B)	
Stage 3: using population from section B (viable cells), identify CD34+ cells.	

Stage 4: Note the number and the percentage of cells CD34+	
Stage 5: calculate the absolute number of cells CD34	Absolute number of CD34+ cells (cells/µl) = (Nº of absolute events in CD34 population / Nº of absolute events in microspheres region) x (Nº of microspheres (indicated in the tube) / Volume of sample used)

The absolute number of the population of CD34 + cells was determined by dividing the absolute number of cells acquired CD34 + by the number of acquired microspheres, and multiplying this result by the concentration of microspheres (concentration of microspheres is indicated on the label of the tube) split by the volume of sample used. If we made some dilution, the final number must be multiplied by the dilution performed.

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[4,5,6].

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

Results obtained for each laboratory are shown below:

SPECIFICITY

Anti CD34 clone 581, was included in the 5th International Workshop of Differentiation Antigens Human Leukocyte, WS code MA276.

The anti-CD45 antibody clone HI30 was included in the 4th workshop of differentiation antigens of human leukocytes with N816112 code.

INTERFERING

Coagulated, fixed or hemolysis samples should not be used.

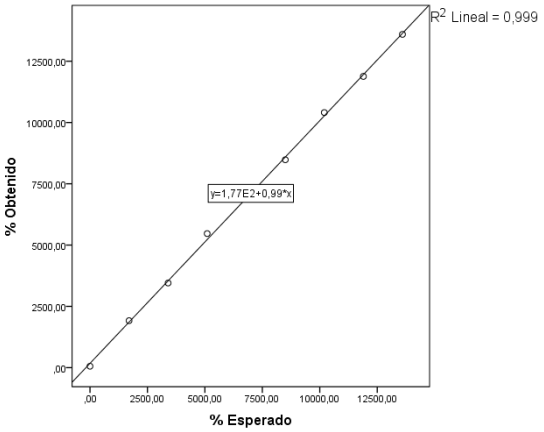
LINEARITY

For analysis of linearity, different dilutions of a positive population (293T cell line transfected with CD34, ref. H34LYS) and a negative population (293T cell line untransfected) were performed keeping constant the total number of cells, the correlation and dependence was analyzed between expected and obtained percentages[8].

Obtained data are shown in the following table

Descriptive statistics

R	R square	Std. Error of the Estimate	Linear regression
1,000[a]	,99 9	142,9315	Y= 0,990x + 176,71



INTERLABORATORY REPEATABILITY AND ACCURACY

The repeatability of Stem Cell Kit was determined by testing 3 samples of commercial control with different levels of MHC with similar values to normal peripheral blood (Low *), bone marrow (Medium *) and blood cord or mobilized blood (H *), performing analysis in two different laboratories over a period of 20 days[7].

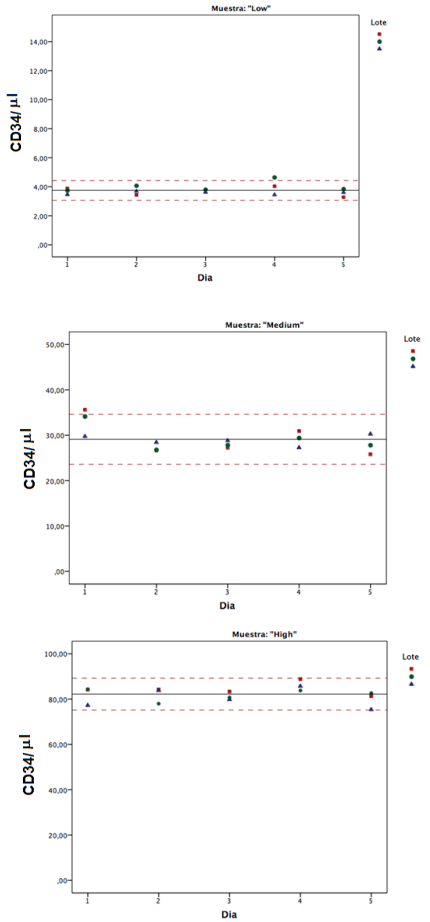
* CD-Chex CD34, Streck

Overall result	Low		Medium		High	
N	50		50		50	
Mean value	0,067		0,0895		2,851	
	SD	CV (%)	SD	CV (%)	SD	CV (%)
Between-lab	1,0079	13,43%	5,7253	9,67%	6,9357	3,85%
Between-day	0,3755	5,00%	2,5918	4,38%	1,4463	0,80%
Whitin-lab	1,0755	14,33%	6,2846	10,61%	7,0849	3,93%

SD: Standard Deviation
CV: Coefficient of Variation

REPRODUCIBILITY BETWEEN BATCHES

To demonstrate reproducibility between batches, 3 control samples with different levels of MHC CD34 +, similar values to normal peripheral blood "Low *" bone marrow "Medium *" and cord blood or mobilized blood "High *" were marked, making analysis with three different batches for 5 days not consecutives[7].
* CD-Chex CD34, Streck
The results for each sample are shown below:



Test result is shown in the following table:

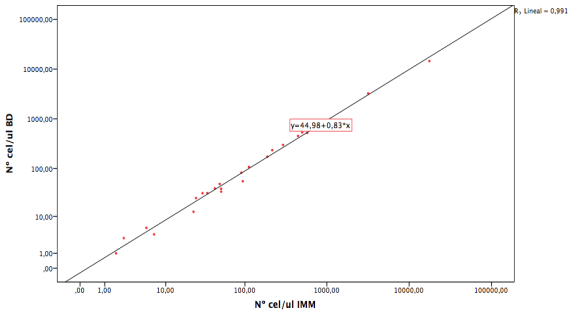
Overall result	High		Medium		Low	
N	50		50		50	
Mean value	0,067		0,0895		2,851	
	SD	CV (%)	SD	CV (%)	SD	CV (%)
Between-Bath	4,4675	6,45%	7,7253	23,31%	0,3376	14,35%

COMPARISON OF MEASUREMENT SYSTEM

To determine the concordance of the results, a comparative measure[9] procedure was performed using a kit reference * in bone marrow samples of healthy and diseased patients, analyzing the absolute number of CD34 + cells.

The following table shows the analysis of the results and the accuracy of the Stem Cell Kit from Immunostep respect to the reference kit *, in a confidence interval of 95% (IC) divided by intervals: low> 10, medium 10 and 100 and high> 100 cells per microliter.

It is a linear representation of obtained data.



Statistical analysis

R	R squared	Standard error of the estimate	Regression line
0,999	,998	133,97930	Y= 0,83x + 44,98

* Becton Dickinson BD™ Stem Cell Enumeration Kit
Catálogue No. 344563

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. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. J Hematother. 1996;5(3):213–226. doi: 10.1089/scd.1.1996.5.213.
2. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. Cytometry. 1998;34(2):61–70.
3. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: CLSI; 2014.
4. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Linearity of Quantitative Measurement Procedures. Second Edition. CLSI guideline EP06-Ed2. Wayne, PA: CLSI; 2020.
5. Clinical and Laboratory Standards Institute (CLSI). Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition. CLSI document EP09-A3. Wayne, PA: CLSI; 2013.

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SURVEILLANCE AND NOTIFICATION

In accordance with Annex I, Section 20.4.1(n) of Regulation (EU) 2017/746, the user is obliged to report any serious incident related to the use of the product.

- **To the Manufacturer:** Please contact our Surveillance Department at vigilancia@immunostep.com
- **To the Competent Authority:** Report via the official channels of the Member State.

EXPLANATION OF SYMBOLS

CE	CE labeling
IVD	*In vitro* diagnostic device
	Manufacturer
	Pay attention to