CD34 QuantiFlowEx Kit

50 tests | Cat. No. **ED7080**

IVD CE 2265

Instructions for Use (EN)

Version: ED7080_IFU_v5_EN

Date of Issue: 24-10-2024

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Temperature limit
CE 2265	CE conformity mark Notified Body ID number		Keep away from sunlight
	Manufacturer		Keep Dry Keep away from rain
UDI	Unique Device Identifier		Caution
	Consult instructions for use	CONC 10×	Concentrated solution (10x)
	Contains sufficient for <n> tests	CONTENTS	Contents
REF	Catalogue number	UK CA	UKCA mark
LOT	Batch code		
	Use by date		

1. Intended Purpose

CD34 QuantiFlowEx Kit is intended for detection and enumeration of total viable hematopoietic stem cells from total viable leukocytes in human blood and tissue samples by flow cytometry.

What is detected and/or measured

The device CD34 QuantiFlowEx Kit detects and measures relative percentages and absolute counts of human viable hematopoietic stem cells (CD34+CD45dim).

Device function

The device is intended for monitoring of hematopoietic stem cell count in peripheral blood, bone marrow and leukapheresis product.

Context of a physiological or pathological state

Accurate enumeration of hematopoietic stem cell (HSCs) count in human blood and tissue samples or grafts for transplantation is necessary for patient management or graft processing [(1)].

Type of assay

Not automated

Quantitative

Type of specimen required

Normal peripheral blood, or mobilized peripheral blood, or leukapheresis product(s), or bone marrow.

Testing population

Not intended for a specific population.

2. Intended user

The device is intended for professional laboratory use only. Not for near-patient testing or self-testing.

Requirements on qualification

Intended user shall have a state-of-the-art expertise in flow cytometry analysis of human cells, standard laboratory techniques, including pipetting skills, safe and proper handling of specimens derived from the human body.

Intended user shall be compliant with standard EN ISO 15189 or other national standards, where applicable.

3. Test principle

The test principle is based on the detection of monoclonal antibody binding to a specific molecule (antigen) expressed by certain human blood cells. Monoclonal antibodies used in the test are labeled with different fluorochromes which are excited by a laser beam from a flow cytometer during acquisition of an antibody-stained blood specimen. Subsequent fluorescence (light emission) from each fluorochrome present on an acquired blood cell is collected and analyzed by the instrument. Fluorescence intensity is directly proportional to the antigen expression density in a cell allowing for separation of different cell subsets.

7-AAD is a cell membrane-impermeant dye that is excluded from viable cells and binds to DNA in non viable cells. Differences in cell fluorescence intensity enable exclusion of non viable cells from analysis.

4. Reagent(s) provided

Contents

The device CD34 QuantiFlowEx Kit is sufficient for 50 tests and is provided with the following reagents:

Staining Reagent (ED7080-1; 1 vial) containing 1 ml of premixed combination of fluorochrome-labeled monoclonal antibodies CD45 FITC and CD34 PE, diluted at optimum concentrations in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide, see Table 1.

7-AAD (ED7080-2; 1 vial) containing 1 ml of 7-Aminoactinomycin D (7-AAD) cell viability dye, diluted at optimum concentration in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.

Lysing Solution (ED7080-3; 1 bottle) containing 15ml of concentrated (10X) ammonium chloride-based, fixative-free buffered solution.

Composition

Table 1 Description and concentrations of active components

Antigen	Flurochrome	Clone	Isotype	Concentration (µg/ml)
CD45	FITC	MEM-28	IgG1	30
CD34	PE	4H11 [APG]	IgG1	35

5. Materials required but not provided

For both Single and Dual Platform Method

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

Process control cells (Streck CD-Chex CD34®, CD34 control – 3 levels , Cat. No. 213337, 213347, 213383 or equivalent lysable cell control with pre-defined CD34 HSC count)

Only for Single Platform Method

Fluorescent cell count standard

- for use with Becton Dickinson cytometers
    - BD Trucount™ Tubes
- for use with Beckman Coulter cytometers
    - Beckman Coulter Flow-Count™ Fluorospheres

6. Equipment required

For both Single and Dual Platform Method

Automatic pipette with disposable tips (20 - 100 µl) for pipetting specimen and reagents

Liquid dispenser or pipette with disposable tips (2 ml) for dispensing erythrocyte lysing solution

Counting beads: TruCount™ Tubes (BD Biosciences; ref. No. 663028), FlowCount Fluorospheres (Beckman Coulter; ref. No. 7547053)

Vortex mixer

Flow cytometer with one laser excitation source (488 nm), detectors for scattered light, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 2.

Table 2 Spectral characteristic of fluorochromes used in the device

Flurochrome	Excitation [nm]	Emission [nm]
FITC	488	525
PE	488	576
7-AAD	488	670

NOTICE: The device was tested on flow cytometers BD FACSCanto™ II and BD FACSLyric™ (BD Biosciences), Navios (Beckman Coulter) and XF-1600™ (Sysmex).

Only for Dual Platform Method

Hematology analyzer (for absolute cell counts) capable of white blood cell (WBC) and lymphocyte count per µl of specimen.

7. Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

Do not freeze.

See Section 10 Procedure (Reagent Preparation) for information about In-Use stability and shelf-life following the first opening, together with the storage conditions and stability of working solutions (where applicable).

8. Warnings, precautions and limitations of use

GHS Hazard Classification

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of infectious materials.

Evidence of deterioration

Normal appearance of the reagents provided is a clear liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Limitation of use

Do not use after the expiry date stated on the product labels.

9. Specimen

Use blood or tissue material collected into specimen receptacle classified as a medical device, with the anticoagulant EDTA, Heparin, or ACD (Acid Citrate Dextrose).

The following specimen can be analyzed using the device:

normal and mobilized peripheral blood, leukapheresis products and bone marrow.

NOTICE: For dual platform analysis determine leukocyte absolute cell count in the collected specimen by a hematology analyzer. The device CD34 QuantiFlowEx Kit alone does not provide enumeration of absolute cell counts.

Process the specimen no later than 24 hours after collection.

Endogenous Interference

Based on scientific literature research endogenous interference sources are identified in Table 3.

Table 3 Endogenous Interference of the device

Endogenous interference	Impact	Reference
Albumin	Albumin may interfere in high concentrations due to its ability to bind as well as to release large quantities of ligands.	2, 3, 4
Bilirubin (icterus) (unconjugated)	Bilirubin may increase fluorescence background of cells due to its high autofluorescence.	5, 6, 7
Cell debris (after lysis)	Cell debris may provide inaccurate cell counts and deplete antibody within the device.	8, 9
Erythrocytes	Insufficient lysis, red blood cells present in sample may yield erroneous cell counting.	6
Hemoglobin	Hemolyzed samples may yield erroneous results.	10
Human anti-Murine antibodies	Monoclonal antibody treatment may yield erroneous results (ability to bind to cell surface antigens).	11, 12, 13, 14, 15, 16
Immunoglobulins	Cannot be washed and may yield erroneous lymphocyte subset count.	8
Rheumatoid factors	Presence of RF does interfere with MIA (multiplex immunoassays).	17
Triglycerides	High circulating levels of lipids may affect flow cytometry analysis of certain blood cell populations.	18

Exogenous Interference

Specimen older than 24 hours may yield erroneous results.

Refrigerated specimen may yield erroneous results.

Incorrect preparation of the erythrocyte lysis solution can lead to erroneous results. Follow the manufacturer's instructions for using the erythrocyte lysis

solution.

10. Procedure

Preparation of reagent(s) provided

Staining Reagent and 7-AAD

No reagent preparation is necessary.

Bring the reagent to the room temperature prior to use. Keep the device primary container dry.

Use the reagent directly from its original primary container.

Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container.

CAUTION: Do not dilute the reagent.

Lysing Solution

Dilute concentrated (10X) erythrocyte lysing solution to the working lysing solution (1X) with deionized water.

CAUTION: The working lysing solution (1X) is stable for **1 day only**. Prepare fresh working lysing solution (1X) each measuring day by mixing 1 part of concentracted (10X) Lysing Solution with 9 parts of deionized water and store in the liquid dispenser or closed container at room temperature.

Preparation of materials required but not provided

For preparation and use of fluorescent cell count standards, follow the manufacturer´s instructions.

Quality control

Use Streck CD-Chex CD34® or equivalent control cells as positive procedural control to ensure proper performance of the device as intended. Streck CD-Chex CD34® provides established values for percent positive and absolute counts of CD34+ HSC.

Stain the control cells using CD34 QuantiFlowEx Kit according to sample processing as specified in the IFU. Verify that the obtained results (% Positive Cells) are within the Expected range reported for the used lot of control cells.

Specimen staining – Single Platform method

1. For each specimen, label a (12 × 75 mm) round bottom test tube with the appropriate sample identification.

   NOTICE: Use BD Trucount™ Tube as a test tube for absolute CD34 stem cell counting.

2. Pipette 20 µl of Staining Reagent into the bottom of the test tube.

3. Pipette 20 µl of 7-AAD into the bottom of the test tube.

4. Pipette 100 µl of well-mixed specimen to the bottom of the test tube using reverse pipetting technique.

   CAUTION: Pipetting accuracy is critical for CD34+ stem cell absolute count enumeration. Therefore, reverse pipetting technique using automatic air displacement pipette shall be used.

   For reverse pipetting sample aspiration, depress pipette knob to its 2nd stop and aspirate sample. Then place the pipette tip containing blood sample near the tube bottom and depress pipette knob to its 1st stop for sample dispensing.

   Avoid pipetting blood on the side of the test tube. If blood smear or droplet remains on the side of the tube, it may not be stained with the reagent or erythrocytes may not be lysed and the test result may not be valid.

5. Vortex and incubate the test tube for 20-30 minutes at room temperature in the dark.

6. Add 2 ml of working lysing solution (1X) to the test tube.

7. Vortex and incubate the test tube for 10 minutes at room temperature in the dark.

8. If BD Trucount™ Tube was not used as a test tube, add 100 µl of Flow Count™Fluorospheres using reverse pipetting technique. Follow manufacturer´s instructions.

9. Acquire the stained sample immediately on the flow cytometer. If the stained sample will not be acquired immediately, cap the test tube, store at 2-8 °C in the dark and analyze within 2 hours.

   CAUTION: Vortex the stained sample immediately before acquisition on the flow cytometer to avoid aggregates.

Specimen staining – Dual Platform method

CAUTION: Determine leukocyte absolute cell count in the collected specimen by a hematology analyzer prior to sample processing.

1. For each specimen, label a (12 × 75 mm) round bottom test tube with the appropriate sample identification.
2. Pipette 20 µl of Staining Reagent into the bottom of the test tube.
3. Pipette 20 µl of 7-AAD into the bottom of the test tube.
4. Pipette 100 µl of well-mixed specimen to the bottom of the test tube using reverse pipetting technique.
5. Vortex and incubate the test tube for 20-30 minutes at room temperature in the dark.
6. Add 2 ml of working lysing solution (1X) to the test tube.
7. Vortex and incubate the test tube for 10 minutes at room temperature in the dark.
8. Acquire the stained sample immediately on the flow cytometer. If the stained sample will not be acquired immediatelly, cap the test tube, store at 2-8 °C in the dark and analyze within 2 hours.

   CAUTION: Vortex the stained sample immediately before acquisition on the flow cytometer to avoid aggregates.

Flow cytometry analysis

The flow cytometer selected for use with the device CD34 QuantiFlowEx Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturers instructions.

If not maintained properly the flow cytometer may produce false results.

Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section 6 (Equipment required).

Set voltages on the fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.

Depending on the sample specimen, acquire at least 300,000 – 1,000,000 events per tube.

Always acquire cell light scatter parameters: Forward Angle Light Scatter (both

Signal Area and Signal Height) and Side (Perpendicular) Light Scatter (both Signal Area and Signal Height).

For **Single Platform** method Set threshold on fluorescence in FITC detector rather than event size for data collection. Threshold on Forward Scatter (event size) may exclude microparticle events of count standard from analysis which would negatively influence CD34+ stem cell percentage enumeration.

For **Dual Platform** method set threshold on Forward Scatter.

Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

> **NOTICE:** Samples with expected low cell viability should be used for preparation of 7-AAD compensation control, e.g. formaldehyde-based lysing solution processed blood cells. Samples with high cell viability provide low number of dead cells. Low dead cell count may negatively influence mean dead cell 7-AAD fluorescence intensity and may produce inadequate compensation.

For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

Data analysis of the CD34 QuantiFlowEx Kit stained specimen

Analyze measured and compensated data using appropriate software. International Society for Hematotherapy and Graft Engineering (ISHAGE) gating protocol (Fig. 1-5) shall be applied for enumeration of percentage of live CD34+ stem cells.

Using 5 parameters (2 light scatter parameters and 3 fluorescent parameters) CD34+ hematopoietic stem cells are identified by a combination of sequential and Boolean gating according to their properties.

True CD34+ stem cells express CD34 and CD45 antigen, however CD45 expression is similar to that of blast cells. Staining intensity is detectable but lower than in e.g. lymphocytes.

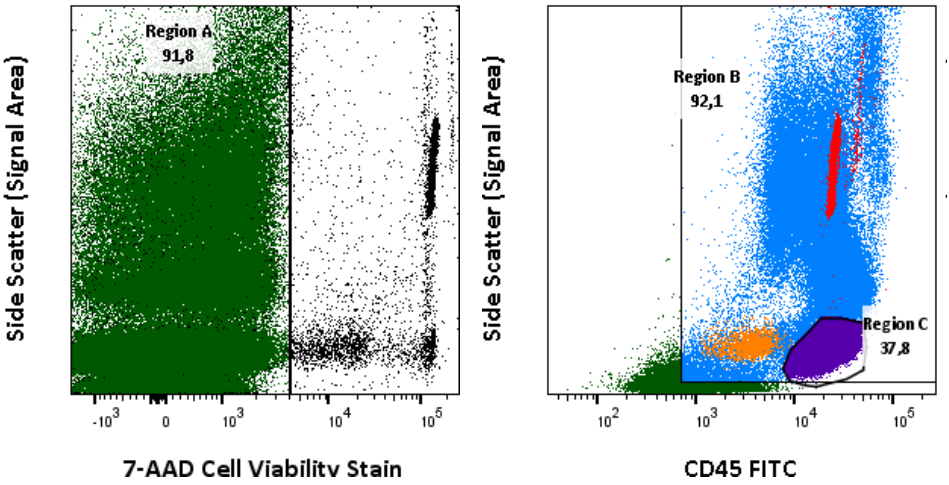
True CD34+ stem cells also provide forward angle light scatter signal similar to blast cells or lymphocytes and exhibit low perpendicular light scatter properties (side scatter).

Figures 1-5 show sequence of logical gating ensuring accurate identification of live CD34⁺ stem cells for accurate percentage enumeration.

First, visualize all events **in a Side Scatter Signal Area (SSC-A) vs. 7-AAD viability staining** dot-plot and place a gate around **live cells (7-AAD negative) as** shown by Region A in the image on the left.

> **NOTICE:** When using stabilized blood control as e.g. Streck CD-Chex CD34® it is strongly advised to check viability Region A and reposition the region if necessary as stabilized blood contains formaldehyde which permeates cell membrane resulting in positive 7-AAD viability dye staining.

Figure 1 The image in the left represents selection of viable population. Image in the right represents all gated events from Regions A, B, C, G (derived from Region F) and I.

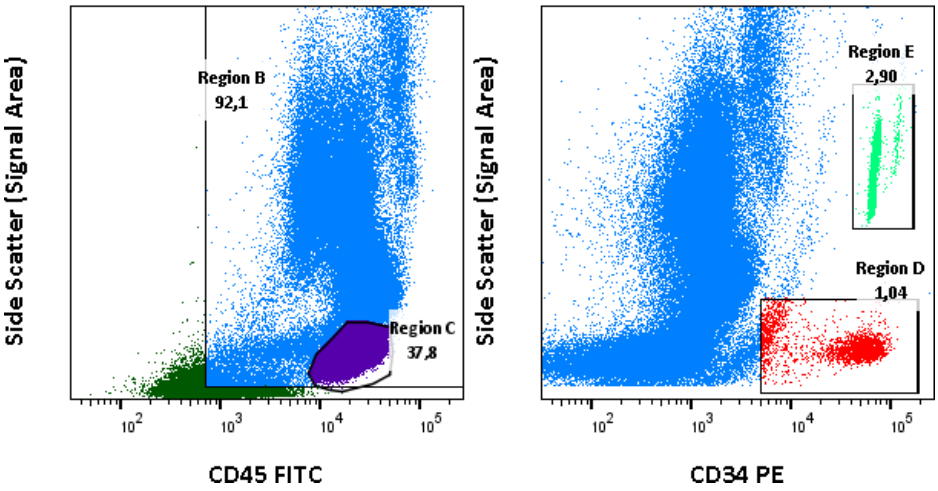


Visualize cells from Region A in a SSC-A vs. CD45 FITC dot-plot and place gate around **leukocytes (Region B)** and a gate around **lymphocytes** represented as **Region C**.

Bring cells from Region B to a SSC-A vs. CD34 PE dot-plot and place gate around **CD34 positive events (Region D)**.

Image on the right shows **all events** including fluorescent microparticles from **Region I** depicted in **Region E**. **Region E** indicates optical and fluorescent properties of fluorescent microparticle count standard present in the BD TruCount™ Tube (only for single-platform method).

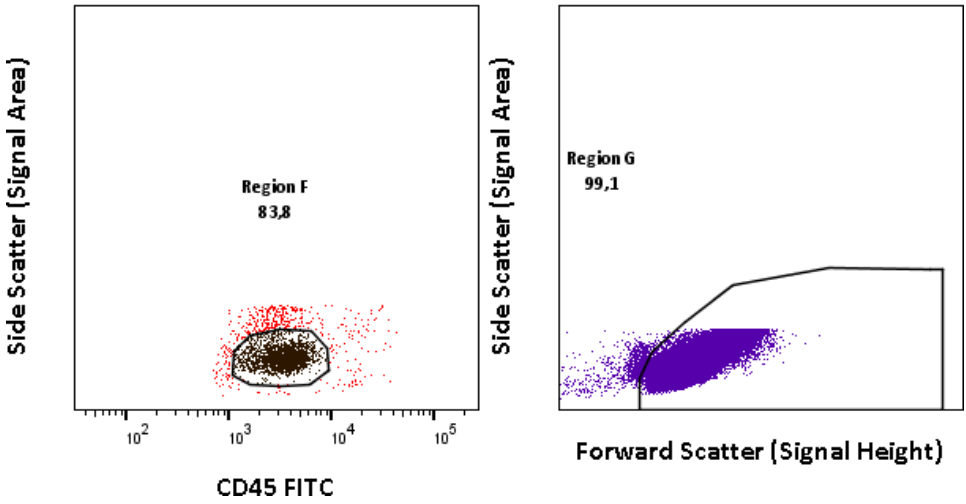
Figure 2 The image in the left represents selection of viable leukocyte (Region B) and lymphocyte (Region C) population. Image in the right visualize viable CD34 positive events (Region D) selected from leukocytes (Region B). For the single platform method, a gate (Region E) can be placed around the fluorescent beads.



Purify CD34 positive events from Region D by placing a **Region F** around CD45 dim-positive cell cluster in SSC-A vs. CD45 FITC dot-plot with events from Region D as shown in the Figure 3 on the left.

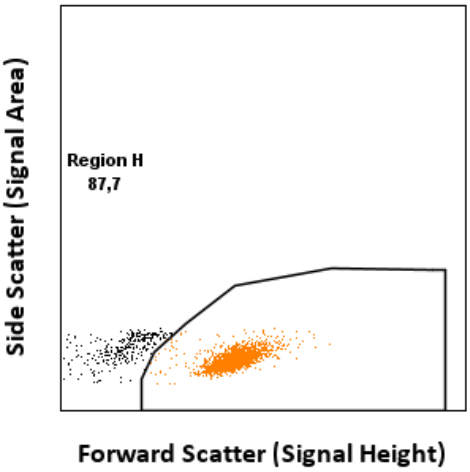
Display $CD45^+$ lymphocytes (**Region C**) in SSC-A vs. Forward Scatter Signal Height (FSC-H) dot-plot. Place a new gate delineating $CD45^+$ lymphocytes from smaller events and debris (**Region G**) as shown in the Figure 3 on the right.

Figure 3 The image on the left visualizes the removal of non-specific CD34 stained cases, i.e. platelets or aggregates. Region F was used to delineate specifically stained cells that are $CD45^{dim}SSCs^{low}$ CD34+. The image on the right visualizes delineating $CD45^+$ lymphocytes (Region G) from Region C.



Copy **Region G** gate delineating lymphocytes from **Fig. 3** (right image) and paste into the SSC-A vs. FSC-H dot-plot containing events from **Region F** and create **Region H**. In order to differentiate the $CD34^+$ stem cells cluster from smaller events and debris. Cells from Region F (Figure 3) found inside the **Region H** gate represent True $CD34^+$ stem cells.

Figure 4 The image represents selection of True CD34+ stem cells (Region H).



Only for Single-platform method:
To ensure that correct gate for fluorescent beads (Region E) is placed, the controlling gates (Region I, J, K in Figure 5 and 6) should be visualized.

Visualize **all events** in CD34 PE vs. CD45 FITC and place regions around fluorescent microparticle count standard delineating microparticles from BD TruCount™ (**Region I**) or microparticles from Beckman Coulter's Flow-Count™ (**Region J**).

NOTICE: Counting bead size and fluorescence properties may differ between different manufacturers.

Figure 5 The image in the left represents size and fluorescent properties of beads BD TruCount™ Tube (Region I). The image in the right represents size and fluorescent properties of beads BC Flow-Count™ Fluorospheres (Region J).

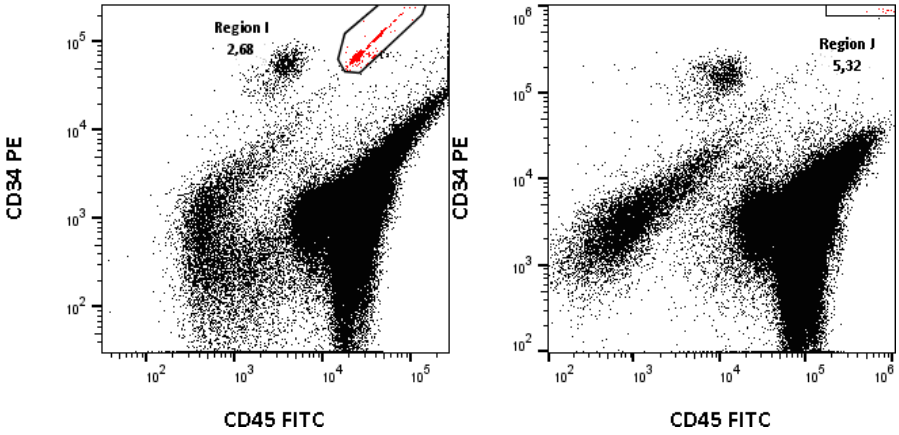
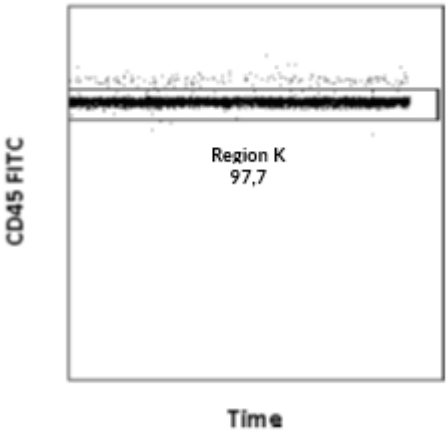


Figure 6 The image visualize all singlet counting beads in time (**Region K**).



NOTICE: Any non-homogeneity in Region K (disturbance in acquisition, decrease in fluorescence) should be considered for a review. Non-homogenous acquisition of events or an acquisition that is not perpendicular to CD45 FITC axis indicates problems with fluidics of a flow cytometer.

Calculation and interpretation of analytical results – Single Platform method

Use equations below for percentage and absolute count enumeration of live CD34⁺ stem cells from all live leukocytes.

Live CD34⁺ stem cell absolute count enumeration per 1 µl of blood material:

$$CD34^{+}Abs = \frac{Region\ H}{Region\ E} \times \frac{P}{V} \times DF$$

Live leukocyte absolute count enumeration per 1 µl of blood material:

$$WBC\ Abs = \frac{Region\ B}{Region\ E} \times \frac{P}{V} \times DF$$

Live CD34⁺ stem cell percentage enumeration from all live leukocytes:

$$\%\ CD34^{+} = \frac{CD34^{+}Abs}{WBC\ Abs} \times 100$$

CD34⁺ Abs	live CD34⁺ stem cell absolute count per 1 µl of blood material
WBC Abs	live leukocyte absolute count per 1 µl of blood material
% CD34⁺	percentage of live CD34⁺ stem cells from all live leukocytes
Region B	number of events in Region B (leukocytes)
Region H	true CD34⁺ stem cells
Region E	number of events in Region E (microparticles)
P	number of microparticles per test (present in the test tube) indicated by microparticle's manufacturer
V	specimen volume – 100 µl
DF	dilution factor (dilution of specimen before staining); DF = 2 means that 1 part of blood material (100 µl) was diluted using 1 part of PBS containing 0.5% BSA (100 µl)

Calculation and interpretation of analytical results – Dual Platform method

Use hematology analyzer for to define leukocyte count per µl of specimen. Refer to hematology analyzer manufacturer's instructions.

Use equations below for percentage and absolute count enumeration of live CD34⁺ stem cells from all live leukocytes.

Live CD34+ stem cell absolute count enumeration per 1 µl of blood material:

$$CD34^{+}Abs = \frac{Region\ H}{Region\ B} \times WBC\ Abs \times DF$$

Live CD34+ stem cell percentage enumeration from all live leukocytes:

$$\%\ CD34^{+} = \frac{Region\ H}{Region\ B} \times 100$$

CD34⁺ Abs	live CD34⁺ stem cell absolute count per 1 µl of blood material
WBC Abs	live leukocyte absolute count per 1 µl of blood material defined using hematology analyzer before applied dilution factor (DF)
% CD34⁺	percentage of live CD34⁺ stem cells from all live leukocytes
Region B	number of events in Region B (leukocytes)
Region H	number of true CD34⁺ stem cells
DF	dilution factor (dilution of specimen before staining); DF = 2 means that 1 part of blood material (100 µl) was diluted using 1 part of PBS containing 0.5% BSA (100 µl)

11. Analytical performance

Specificity

The antibody MEM-28 recognizes all leukocyte isoforms of human CD45 (Protein tyrosine phosphatase receptor type C). Specificity of the antibody has been confirmed by HLDA workshop (HLDA III workshop [19]).

The antibody 4H11[APG] recognizes Class III epitope of human CD34 antigen (Mucosialin). Specificity of the antibody has been confirmed by HLDA workshop (HLDA VI workshop [20]).

Accuracy

Accuracy of the method was determined as a comparison of the device CD34 QuantiFlowEx Kit with well-documented accredited clinical laboratory in-house dual-platform method (cocktail of single color conjugated antibodies from different manufacturers combined with amonium chloride based lysing solution) by parallel staining of 75 blood or tissue samples analysed by both BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer (table 4, 5, 6). Linear regression analysis parameters are provided in Table 4 - 6.

Table 4 Linear regression analysis for CD34+ stem cells in peripheral blood (comparison of the device CD34 QuantiFlowEx Kit with accredited clinical laboratory in-house method) analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison ED7080 with accredited method						
Peripheral blood						
Target population	**Unit**	**n**	**Slope**	**Intercept**	**r^2**	**Range**
CD34⁺CD45dim	%	30	0.9743	-0.0005	0.9967	0.02 - 2.22
	cells/µl	30	0.9757	-0.4106	0.9947	0.24 - 468

Table 5 Linear regression analysis for CD34+ stem cells in leukapheresis products (comparison of the device CD34 QuantiFlowEx Kit accredited clinical laboratory in-house method) analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison ED7080 with accredited method						
Leukapheresis products (PBSC)						
Target population	**Unit**	**n**	**Slope**	**Intercept**	**r^2**	**Range**
$CD34^+CD45dim$	%	25	0.9999	-0.0061	0.9925	0.81 - 10.56
	cells/µl	25	0.9844	45.762	0.9918	1392 - 17497

Table 6 Linear regression analysis for CD34+ stem cells in bone marrow (comparison of the device CD34 QuantiFlowEx Kit accredited clinical laboratory in-house method) analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison ED7080 with accredited method						
Bone marrow						
Target population	**Unit**	**n**	**Slope**	**Intercept**	**r^2**	**Range**
$CD34^+CD45dim$	%	20	0.9385	0.0467	0.9954	0.24 - 3.14
	cells/µl	20	1.028	-4.1351	0.9991	47 - 1708

Linearity

The linearity of the method was verified on “Buffy Coat” blood derivative of healthy blood donor spiked with 11 consecutive (serial; 2-fold) dilutions of CD34+ cells (KG-1) in 1 day by 1 operator analyzed by BD FACSCanto™ II flow cytometer. Linear regression was used for evaluation of expected value against mean recovered value at each dilution. Linearity range is listed in table 7.

Table 7 Linearity of the device on BD FACSCanto™ II

BD FACSCanto™ II					
Target population	**Unit**	**Slope**	**Intercept**	**r^2**	**Range**
$CD34^+CD45dim$	cells/µl	1.0648	4.4804	1.0000	3.64 – 2862

Repeatability

The repeatability of the assay was measured on ten blood samples in hexaplicates. Samples were analyzed using BD FACSLyric™ flow cytometer and Sysmex XF-1600™ flow cytometer. Coefficients of variation (CV) are provided in the tables below (Table 8 and 9).

Table 8 Repeatability of the device on BD FACSLyric™

BD FACSLyric™					
Target population	**Unit**	**n**	**Evaluated range of value**	**SD**	**CV (%)**
$CD34^+CD45dim$	%	10	0.03-0.07	0.0035	7.2
	cells/µl	10	8-20	1.0	7.2

Table 9 Repeatability of the device on Sysmex XF-1600™

Sysmex XF-1600™					
Target population	**Unit**	**n**	**Evaluated range of value**	**SD**	**CV (%)**
$CD34^+CD45dim$	%	10	0.03-0.07	0.0047	9.4
	cells/µl	10	7-20	1.2	9.4

Reproducibility

The reproducibility of the assay was measured on stabilized blood sample (CD-Chex CD34, Level 3) under the same conditions for 15 days. Samples were analyzed using BD FACSLyric™ flow cytometer and Sysmex XF-1600™ flow cytometer. Coefficients of variation (CV) are given in the tables below (Table 10 and 11).

Table 10 Reproducibility of the device on BD FACSLyric™

Reproducibility – BD FACSLyric™						
Sample Type	**Expected range (%)**	**Expected mean value (%)**	**Obtained mean value (%)**	**SD**	**CV (%)**	**Measured range of values**
CD-Chex CD34, Level 3	1.35 – 1.95	1.65	1.67	0.06	3.6	1.46-1.70

Table 11 Reproducibility of the device on Sysmex XF-1600™

Reproducibility – Sysmex XF-1600™						
Sample Type	**Expected range (%)**	**Expected mean value (%)**	**Obtained mean value (%)**	**SD**	**CV (%)**	**Measured range of values**
CD-Chex CD34, Level 3	1.35 – 1.95	1.65	1.54	0.04	2.8	1.48-1.62

NOTICE: For flow cytometry analysis following flow cytometers including software version were used:

BD FACSCanto™ II	BD FACSDiva Software – version 8.0.2
BD FACSLyric™	BD FACSSuite Software – version 1.5
Sysmex XF-1600™	IPU Software – version 0(0.09-00)
Navios EX	Navios Ex Software v2.0

For absolute cell counts using the dual platform method hematology analyzer with the following specifications was used:

Sysmex XN-1000™	IPU Software – version 00-22(164)

For evaluation of measured data following analysis platform was used:
FlowJo™ (Becton, Dickinson and Company) - version 10.9.0

12. Clinical performance

Clinical data was collected at a clinical site. Clinical performance of the device ED7080 was determined by comparison of CD34 QuantiFlowEx Kit with accredited clinical laboratory in-house method. 75 samples, including peripheral blood, leukapheresis products and bone marrow samples were tested.
The difference between the methods was less than 10% in 67 out of 75 determinations, meaning that the results obtained by both methods were largely the same. The relative differences between the methods ranged from -13 % to +11 %. These data were further compared using linear regression analysis and Bland-Altmann assessment of agreement between methods (relative difference in counts), which showed showed very good agreement between the methods.
The average value of relative differrences in count between two methods is 1% and a standard error of 6%.
The guidelines for CD34+ HSC determination suggest comparing the results with maximum acceptable relative difference between duplicates up to 10% [21].
According to those recommendations these methods provide data that can be looked at as equal to each other.

13. Expected values

The device is intended for detection and enumeration of total viable hematopoietic stem cells and does not establish any diagnosis by itself where normal range of values could be established.
For three specimen types, value ranges obtained from clinical study are presented in Section 11 (Analytical performance), part Accuracy.

14. Limitations

The device is not intended for identification and enumeration of mesenchymal, neural, epithelial and skin stem cells.

Sample with very high WBC count should be diluted before staining in PBS to obtain leukocyte count less than 20 x 10^3 cell/µl [(22)].

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16. Summary of safety and performance

The summary of safety and performance will be available in the Eudamed database at https://ec.europa.eu/tools/eudamed/#/screen/home. Until then the summary of safety and performance is available upon request.

17. Use of Third Party Trademarks

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18. Revision History

Version 5, ED7080_IFU_v5

New IFU layout according to IVDR (EU) 2017/746 requirements. Addition of the Notified Body ID number. Added new chapter number 16. Summary of safety and performance. Modification of the gating strategy text, recommendations for single-platform gating have been made. Clarification of the term dilution factor. Changes in chapters 11., 12., 14. and 15. (addition of analytical and clinical data).

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NOTICE: Any serious incident that has occured in relation to the device shall be reported to the manufacturer and the local competent authority.