CD34 QuantiFlowEx Kit

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

IVD CE

Instructions for Use (EN)

Version: ED7080_IFU_v4_EN

Date of Issue: 27-07-2023

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Temperature limit
CE	CE marking of conformity		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)
Σ	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™ (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.

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 12 x 75 mm test tube.

3. Pipette 20 µl of 7-AAD into the bottom of the 12 x 75 mm 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 12 x 75 mm test tube.
3. Pipette 20 µl of 7-AAD into the bottom of the 12 x 75 mm 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.

Figure 1 Image in the left represents all gated events from Regions A, B, C, G (derived from Region F) and I.

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 right.

Note: 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.

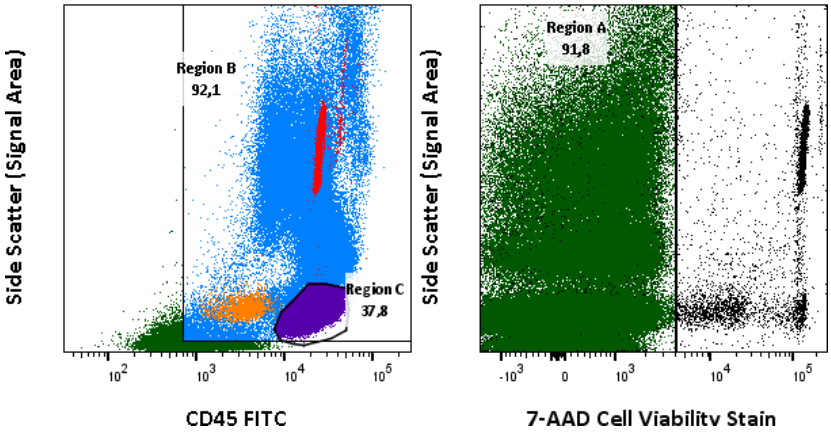


Figure 2 Visualize cells from Region A in a SSC-A vs. CD45 FITC dot-plot and place gate around **leukocytes (Region B)** and a gate around **lymphocytes** represented as **Region C**. Bring cells from Region B to a SSC-A vs. CD34 PE dot-plot and place gate around **CD34 positive events (Region D)**. 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.

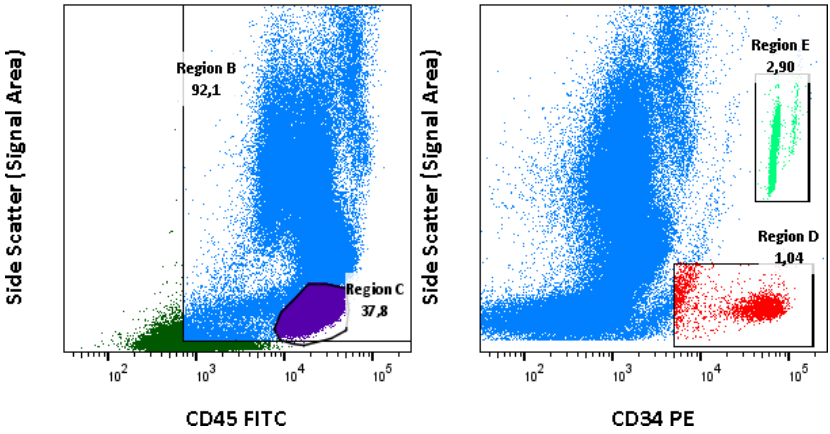


Figure 3 Visualize **all events** in CD34 PE vs. CD45 FITC and place regions around fluorescent microparticle count standard delineating microparticles from BD TruCount™ acquired on BD FACSCanto™ cytometer (**Region I**) and microparticles from Beckman Coulter's Flow-Count™ acquired on a Beckman Coulter Navios™ cytometer (**Region J**).

Note 1: Counting bead size and fluorescence properties may differ between different manufacturers. **Fig. 3** represents size and fluorescent properties of beads present in BD TruCount™ Tube or Beckman Coulter Flow-Count™ Fluorospheres.

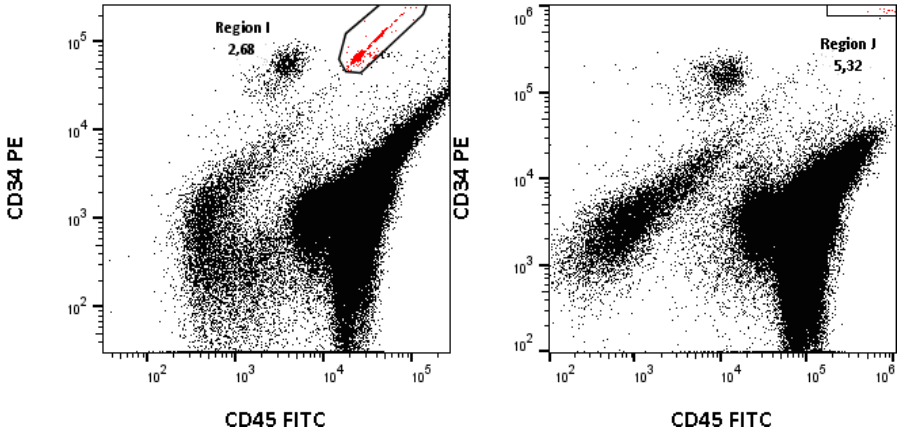


Figure 4 Purify CD34 positive events from Region D by placing a **Region F** around CD45 dim-positive cell cluster in SSC-A vs CD45 FITC dot-plot with events from Region D as shown in the image on the left.

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 image on the right.

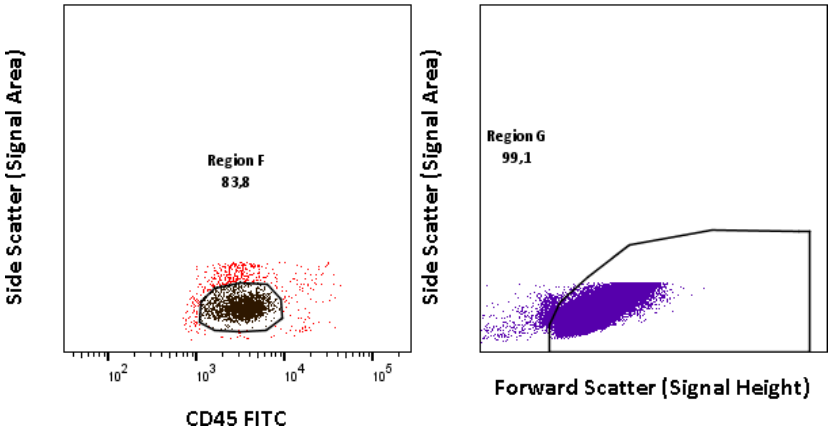
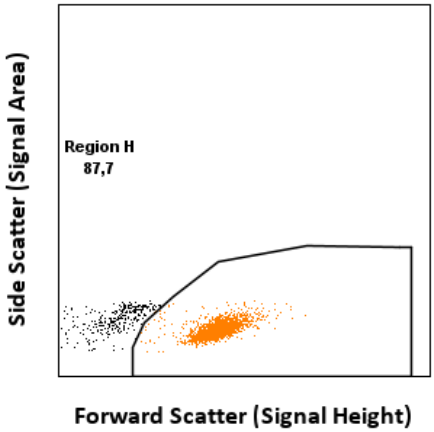


Figure 5 Copy **Region G** gate delineating lymphocytes from **Fig. 4** (right image) and paste the region (**Region H**) into SSC-A vs FSC-H dot-plot containing events from **Region F** in order to differentiate the $CD34^+$ stem cells cluster from smaller events and debris. Cells from Region F (Figure 4) found inside the **Region H** gate represent True $CD34^+$ stem cells.



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

% 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 [(2)]).

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 [(3)]).

Accuracy

Accuracy of the method was determined as a comparison of the device CD34 QuantiFlowEx Kit with similar product available on the market (BD Stem Cell Enumeration Kit, cat. n. 344563) by parallel staining of 34 blood or tissue samples analysed by both BD FACSLyric™ flow cytometer and Sysmex XF-1600 flow cytometer (table 3, 4) together with other well-documented accredited method by parallel staining of 75 blood or tissue samples analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer (table 5, 6, 7).

Linear regression analysis parameters are provided in Table 3 - 7.

Table 3 Linear regression analysis for CD34+ stem cells in donors (comparison of the device CD34 QuantiFlowEx Kit with IVD product BD Stem Cell Enumeration Kit. (cat. n. 344563) analysed by BD FACSLyric™ flow cytometer.

Comparison with BD Stem Cell Enumeration Kit						
BD FACSLyric™						
Target population	**Unit**	**n**	**Slope**	**Intercept**	**r^2**	**Range**
CD34⁺CD45dim	%	34	0.9313	0.0773	0.9602	0.12 - 1.22
	cells/µl	34	1.0152	-8.2612	0.9977	6 - 2137

Table 4 Linear regression analysis for CD34+ stem cells in donors (comparison of the device CD34 QuantiFlowEx Kit with IVD product BD Stem Cell Enumeration Kit (cat. n. 344563) analysed by Sysmex XF-1600 flow cytometer.

Comparison with BD Stem Cell Enumeration Kit						
Sysmex XF-1600						
Target population	**Unit**	**n**	**Slope**	**Intercept**	**r^2**	**Range**
$CD34^+CD45dim$	%	34	0.9600	0.0473	0.9572	0.22 - 1.28
	cells/µl	34	1.0233	-2.3868	0.9977	22 - 5035

Table 5 Linear regression analysis for CD34+ stem cells in peripheral blood (comparison of the device CD34 QuantiFlowEx Kit accredited clinical laboratory in-house method – a cocktail of single color conjugated antibodies from different manufacturers combined with amonium chloride based lysing solution analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison 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 6 Linear regression analysis for CD34+ stem cells in leukapheresis products (comparison of the device CD34 QuantiFlowEx Kit accredited clinical laboratory in-house method – a cocktail of single color conjugated antibodies from different manufacturers combined with amonium chloride based lysing solution analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison 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 7 Linear regression analysis for CD34+ stem cells in bone marrow (comparison of the device CD34 QuantiFlowEx Kit accredited clinical laboratory in-house method – a cocktail of single color conjugated antibodies from different manufacturers combined with amonium chloride based lysing solution analysed by BD FACSCanto™ II flow cytometer or Beckman Coulter Navios flow cytometer.

Comparison 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™ flow cytometer. Linear regression was used for evaluation of expected value against mean recovered value at each dilution. Linearity range is listed in table 8.

Table 8 Linearity of the device on BD FACSCanto™

BD FACSCanto™					
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 9 and 10).

Table 9 Repeatability of the device on BD FACSLyric™

BD FACSLyric™					
Target population	**Unit**	**n**	**Average**	**SD**	**CV (%)**
$CD34^+CD45dim$	%	10	1.21	0.051	4.31
	cells/µl	10	334	109	33

Table 10 Repeatability of the device on Sysmex XF-1600

Sysmex XF-1600					
Target population	**Unit**	**n**	**Average**	**SD**	**CV (%)**
$CD34^+CD45dim$	%	10	1.6	0.103	4.67
	cells/µl	10	448	166	37

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 11 and 12).

Table 11 Reproducibility of the device on BD FACSLyric™

Reproducibility – BD FACSLyric™				
Target population	**Expected mean value (%)**	**Obtained mean value (%)**	**SD**	**CV (%)**
$CD34^+CD45dim$	1.65	1.67	0.0004	2.42

Table 12 Reproducibility of the device on Sysmex XF-1600

Reproducibility – Sysmex XF-1600				
Target population	**Expected mean value (%)**	**Obtained mean value (%)**	**SD**	**CV (%)**
$CD34^+CD45dim$	1.65	1.66	0.0004	2.66

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. Based on data obtained, 100% agreement in subsequent management of the patient between methods was found.

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. Assessment of specimen quality depends on the intended application.

For three specimen types, value ranges obtained from clinical study are presented in Section 11 (Analytical performance), part Accuracy.

14. Interfering substances and limitations

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

15. References

1) Sutherland DR, et. al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother. 1996 Jun;5(3):213-26. doi: 10.1089/scd.1.1996.5.213.
2) McMichael AJ, et al. eds. Leucocyte Typing III. White Cell Differentiation Antigens. Oxford: Oxford University Press, 1987.
3) Kishimoto T, et al. eds. Leucocyte Typing VI. New York: Garland Publishing, Inc., 1997.
4) Whitby A, et. al. ISHAGE protocol: are we doing it correctly? Cytometry B Clin Cytom. 2012 Jan;82(1):9-17. doi: 10.1002/cyto.b.20612. Epub 2011 Sep 13. PMID: 21915992.

16. Trademarks

17. Revision History

Version 4, ED7080_IFU_v4

Umbilical cord blood was deleted from the Device function, in the sections Materials required but not provided and Equipment required (Chapters 5 and 6) there was a division for single- and dual-platform method, correction of errors in the gating strategy, changes in chapters 11., 12. and 13. (addition of analytical and clinical data), citation added.

CD34 stem cell gating strategy has been revised. True CD34+ stem cells (Region H) have been fully specified.
Page 15: absolute count calculation formula correction.

18. Manufacturer

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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.