DryFlowEx TBNK 6-color

50 tests | Cat. No. **ED7736**

IVD CE

Instructions for Use (EN)

Version: ED7736_IFU_v2_EN

Date of Issue: 13-02-2024

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		Do not re-use
	Contains sufficient for <n> tests	TUBE	Contains <n> tubes for single use test
REF	Catalogue number	CONTENTS	Contents
LOT	Batch code	UK CA	UKCA mark
	Use by date		

1. Intended Purpose

DryFlowEx TBNK 6-color is intended for detection and enumeration of lymphocyte populations and subsets in human whole blood by flow cytometry.

What is detected and/or measured

The device DryFlowEx TBNK 6-color detects and measures relative percentages and absolute counts of human T cells (CD3+), B cells (CD3-CD19+), NK cells (CD3-CD16+56+), helper/inducer (CD3+CD4+) and suppressor/cytotoxic (CD3+CD8+) T cell subsets.

Device function

The device is intended for use in the immunological assessment of normal patients, and might aid to diagnosis of patients having, or suspected of having, immune deficiency.

Context of a physiological or pathological state

Frequencies of lymphocyte populations measured by the device can be affected by various pathological conditions and evaluation of their percentages and counts can be used in the assessment of:

- human immunodeficiency virus (HIV) infection progression [1, 5, 9, 11]
- hereditary immunodeficiencies [3, 4, 13, 14, 17, 19]
- autoimmune diseases [6, 8]
- defects in innate immune defense [15, 16]

Type of assay

Not automated

Quantitative

Type of specimen required

Human anticoagulated peripheral whole blood specimen

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.

4. Reagent(s) provided

Contents

The device DryFlowEx TBNK 6-color is sufficient for 50 tests and is provided with the following reagent:

TBNK 6-color ED7736-1 (10 pouches). Each pouch consists of 5 capped single-use tubes containing premixed combination of fluorochrome-labeled reagents dried with the stabilizing ingredients as a layer at bottom of the test tubes (12 x 75 mm), see Table 1.

TBNK 6-color Compensation Set ED7736-2 (1 pouch) containing 6 capped single-use tubes, each containing single fluorochrome-labeled reagent (see Table 1) dried with the stabilizing ingredients as a layer at the bottom of the tube (12 x 75 mm).

> **CAUTION**: TBNK 6-color Compensation Set is intended for the compensation setup only. Single fluorochrome-labeled reagents (see Table 1) allow easy and accurate compensation procedure.

Composition

Table 1 Description of the TBNK 6-color active ingredients

Antigen	Flurochrome	Clone	Isotype
CD3	FITC	UCHT1	IgG1
CD16	PE	3G8	IgG1
CD56	PE	LT56	IgG2a
CD45	PerCP-Cy™5.5	MEM-28	IgG1
CD4	PE-Cy™7	MEM-241	IgG1
CD19	APC	LT19	IgG1
CD8	APC-Cy™7	LT8	IgG1

5. Materials required but not provided

Erythrocyte lysing solution EXCELLYSE Easy, Cat. No. ED7066

Process control cells (Streck CD-Chex Plus®, Cat. No. 213323 or equivalent lysable cell control)

Phosphate buffered saline (1X PBS), pH 7.2 – 7.4

6. Equipment required

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

Liquid dispenser or pipette with disposable tips (0.5 – 2 ml) for dispensing erythrocyte lysing solution

Liquid dispenser or pipette with disposable tips (0.2 – 0.5 ml) for dispensing PBS

Vortex mixer

Centrifuge with appropriate rotor adaptors for 12 x 75 mm round bottom tubes

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

Flow cytometer with two laser excitation sources (488 nm and ~635 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 use in the device

Flurochrome	Excitation [nm]	Emission [nm]
FITC	488	525
PE	488	576
PerCP-Cy™5.5	488	695
PE-Cy™7	488	780
APC	630 – 640	660
APC-Cy™7	630 - 640	780

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

7. Storage and handling

Store at 20-30 °C.

Avoid prolonged exposure to light.

Keep dry.

CAUTION: Moisture sensitive product. Do not open the foil pouch until the first use.

After the first opening, thoroughly reseal the foil pouch with the zip-lock for storage of the remaining unused tubes.

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 reagent provided is a transparent dried layer at the bottom of the tube. Do not use the reagent if you observe any change in appearance, for example presence of moisture inside the tube.

Limitation of use

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

Do not re-use test tubes.

9. Specimen

Use venous peripheral blood collected in specimen receptacle classified as a medical device, with the anticoagulant EDTA.

NOTICE: Determine WBC absolute cell count and lymphocyte count in the collected blood specimen by a hematology analyzer. The DryFlowEx TBNK 6-color alone does not provide enumeration of absolute cell counts.

Blood specimen with WBC count exceeding $40x10^3$ cells/µl will require dilution with 1X PBS before sample processing.

Process the blood specimen no later than 24 hours after collection. Store the specimen at laboratory temperature (20 – 25°C). Do not refrigerate the specimen.

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.	20, 21, 37
Bilirubin (icterus) (unconjugated)	Bilirubin may increase fluorescence background of cells due to its high autofluorescence.	24, 26, 30
Cell debris (after lysis)	Cell debris may provide inaccurate cell counts and deplete antibody within the device.	23, 27
Erythrocytes	Insufficient lysis, red blood cells present in sample may yield erroneous cell counting.	28
Hemoglobin	Hemolyzed samples may yield erroneous results.	25
Human anti-Murine antibodies	Monoclonal antibody treatment may yield erroneous results (ability to bind to cell surface antigens).	22, 32, 33, 34, 35, 36
Immunoglobulins	Cannot be washed in single-platform method and may yield erroneous lymphocyte subset count.	23
Rheumatoid factors	Presence of RF does interfere with MIA (multiplex immunoassays).	29
Triglycerides	High circulating levels of lipids may affect flow cytometry analysis of certain blood cell populations.	31

Exogenous Interference

Specimen older than 24 hours may yield erroneous results.

Refrigerated specimen may yield erroneous results.

Improper erythrocyte lysing solution preparation may yield erroneous results. Follow instructions for use of the device.

10. Procedure

Preparation of reagent(s) provided

TBNK 6-color

No reagent preparation is necessary, supplied in test tubes for single use only.

CAUTION: Moisture sensitive product. Do not open the foil pouch until the first use.

	Each pouch consists of 5 capped single-use TBNK 6-color tubes. After each opening, thoroughly reseal the foil pouch with the zip-lock for storage of the remaining unused tubes. After the first opening, use remaining TBNK 6-color tubes within 30 days.

Preparation of materials required but not provided

Dilute concentrated erythrocyte lysing solution with deionized water according to the manufacturer´s instructions. Diluted (1X) erythrocyte lysing solution is stable for 1 month when stored in a liquid dispenser or closed container at room temperature.

Compensation setup

Acquire Compensation Set tubes using the same flow cytometer set-up, prior to the analysis of TBNK 6-color stained tubes.

CAUTION: TBNK 6-color and TBNK 6-color Compensation Set require the same type of specimen.

TBNK 6-color compensation tubes

1. Pipette 50 µl of well-mixed blood specimen into the bottom of each single-color compensation tube.
2. Vortex vigorously for 7-10 seconds and incubate for 20 minutes at room temperature in the dark.
3. Add 1 ml of diluted (1X) erythrocyte lysing solution to each compensation tube.
4. Vortex and incubate for 10 minutes at room temperature in the dark.
5. Centrifuge for 5 minutes at 300×g, discard supernatant and resuspend the cell

pellet in 0.2 ml of 1X PBS.

6. Set voltages on 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.
7. Acquire the stained sample immediately using flow cytometer.

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.

Set the gates for positive and negative populations for each compensation tube according to the Figure 1.

Calculate compensation matrix either in cytometer software developed by manufacturer or software dedicated for offline cytometry data analysis. Use this compensation matrix for all test tubes of this lot of TBNK 6-color.

CAUTION: Once set for the specific TBNK 6-color lot, do not change fluorescent detectors settings in order to retain the same compensation matrix acquisition settings and compensation results.

Quality control

Use Streck CD-Chex Plus® or equivalent stabilized blood as positive procedural control to ensure proper performance of the device as intended. Streck CD-Chex Plus® provides established values for percent positive and absolute counts of T cells, B cells, granulocytes, monocytes and NK cells, including two clinically relevant levels of CD4+ cells.

Stain the control blood using TBNK 6-color test tube 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

1. Label TBNK 6-color tube with the appropriate sample identification.
2. Pipette 50 µl of well-mixed blood specimen into the bottom of the TBNK 6-color tube.

   CAUTION: 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.
3. Vortex vigorously for 7 – 10 seconds and incubate the test tube for 20 minutes at room temperature in the dark.

CAUTION: Shortening the vortex time may affect the test results.

4. Add 1 ml of diluted (1X) erythrocyte lysing solution to TBNK 6-color tube.
5. Vortex and incubate for 10 minutes at room temperature in the dark.
6. Centrifuge the TBNK 6-color tube for 5 minutes at 300×g.
7. Discard supernatant without disturbing the cell pellet and add 0.2 ml of 1X PBS to the test tube.
8. Vortex shortly to resuspend the cell pellet.
9. Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediatelly, store at 2-8 °C in the dark and analyze within 24 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 DryFlowEx TBNK 6-color 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.

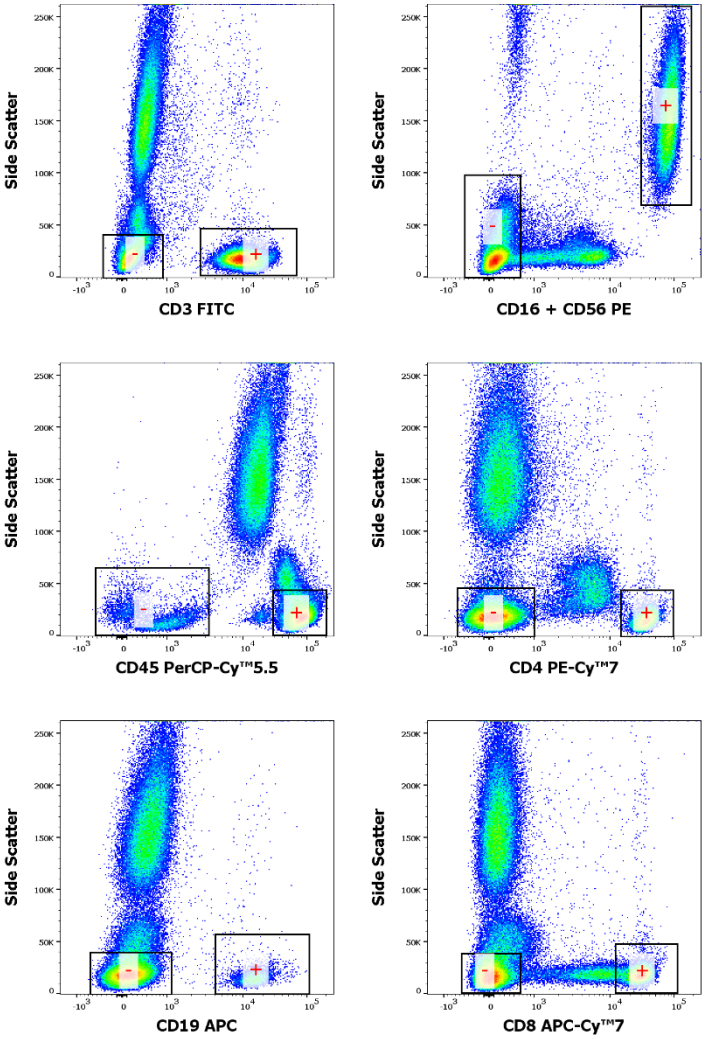
Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section 6 Equipment required.

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™).

Analysis of the compensation tubes

Visualize non-compensated data for each compensation tube in a side-scatter (SSC) versus “fluorochrome to be compensated” dot-plot. Set the gates for positive (+) and negative (-) populations as shown in Figure 1.

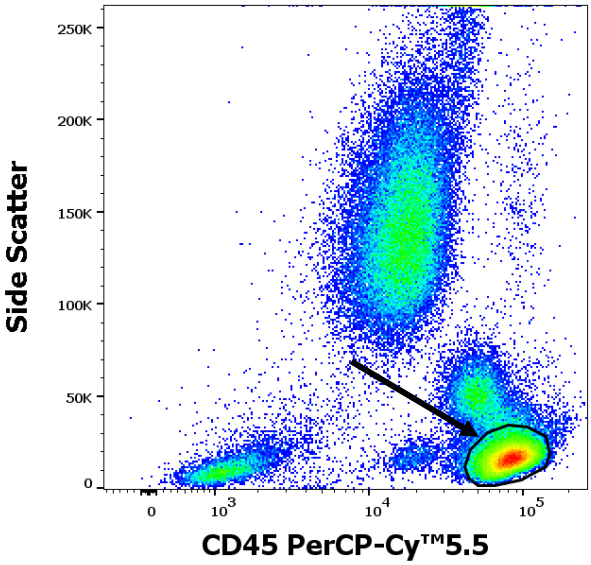
Figure 1 Identification of positive (+) and negative (-) events in compensation tubes (data acquired on BD FACSCanto™ II)



Analysis of the TBNK 6-color stained specimen

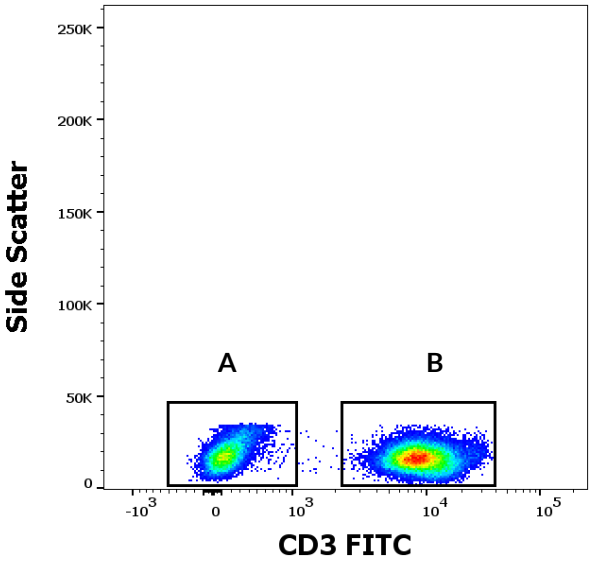
Visualize compensated data in a side-scatter (SSC) versus CD45 PerCP-Cy™5.5 plot. Set the gate for CD45+ lymphocyte population as shown in Figure 2.

Figure 2 Delineation of CD45+ lymphocyte population (data acquired on BD FACSCanto™ II)



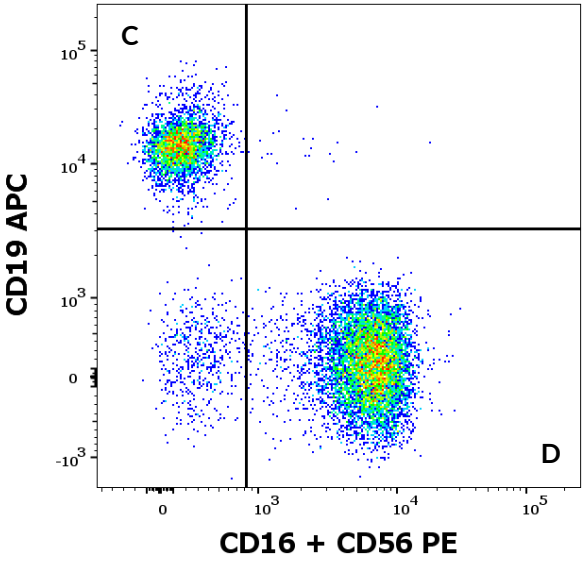
Plot the gated CD45+ lymphocytes in a side-scatter (SSC) versus CD3 FITC plot as shown in Figure 3. Separate CD3+ and CD3- lymphocytes using appropriate gates. Calculate the percentage of T cells (CD3+; region B on the Figure 3) from all lymphocytes.

Figure 3 Separation of CD3+ and CD3- lymphocytes (data acquired on BD FACSCanto™ II)



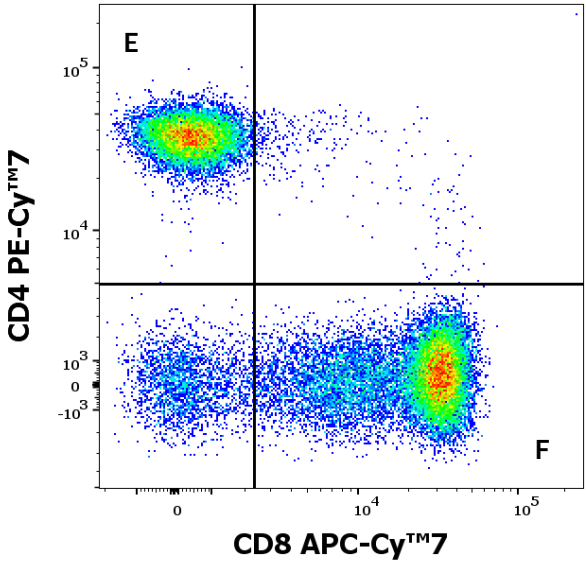
Plot the gated CD3- lymphocytes (region A on the Figure 3) as CD19 APC versus CD16+CD56 PE as shown in Figure 4. Set appropriate gates and calculate the percentage of B cells (CD16-CD56-CD19+; region C on the Figure 4) and natural killer (NK) cells (CD16+CD56+CD19-; region D on the Figure 4) from all lymphocytes.

Figure 4 CD3- lymphocytes in a dot-plot CD19 APC vs. CD16+CD56 PE (data acquired on BD FACSCanto™ II)



Plot the gated T cells (CD3+; region B on the Figure 3) as CD4 PE-Cy™7 versus CD8 APC-Cy™7 as shown in Figure 5. Set appropriate gates and calculate the percentage of helper/inducer T cells (CD4+CD8-; region E on the Figure 5) and suppressor/cytotoxic T cells (CD4-CD8+; region F on the Figure 5) from all lymphocytes.

Figure 5 CD3+ lymphocytes in a dot-plot CD4 PE-Cy™7 vs. CD8 APC-Cy™7 (data acquired on BD FACSCanto™ II)



Calculation and interpretation of analytical results

To have absolute counts, use the absolute lymphocyte count as determined by a hematology analyzer. Refer to hematology analyzer manufacturer's instructions. Use the equations below for absolute count enumeration of required lymphocyte subset.

$$\text{A x } \frac{B\ (\%)}{100\ (\%)} = Absolute\ count\ of\ required\ lymphocyte\ subset$$

A = absolute lymphocyte count (data from hematology analyzer; cells / µl)

B = relative percentages of required lymphocyte subset from all lymphocytes (data from flow cytometer; %)

11. Analytical performance

Specificity

The antibody UCHT1 recognizes an extracellular epitope on CD3 antigen of the TCR/CD3 complex on mature human T cells. The UCHT1 antibody reacts with the epsilon chain of the CD3 complex. Specificity of the antibody has been confirmed by HCDM Council (HLDA I[2], HLDA III[12], and HLDA VI[7] workshop)

The antibody MEM-241 recognizes human CD4 antigen (T cell surface glycoprotein CD4). Specificity of the antibody has been confirmed by HCDM Council (HLDA VIII workshop).

The antibody LT8 recognizes human CD8 antigen (disulfide-linked dimer expressed as two CD8 alpha chain homodimers or CD8 alpha/beta chain heterodimers). Specificity of the antibody has been confirmed by HLDA workshops (HLDA V workshop[18] and HLDA VII workshop[10]).

The antibody 3G8 recognizes human CD16 antigen (low affinity immunoglobulin type III Fc-gamma receptor). Specificity of the antibody has been confirmed by HLDA workshop (HLDA V workshop[18]).

The antibody LT56 recognizes the leukocyte isoform of human CD56 antigen (Neural cell adhesion molecule 1). Specificity of the antibody has been confirmed by HCDM Council (HLDA X workshop).

The antibody LT19 recognizes human CD19 antigen (B cell transmembrane glycoprotein CD19). Specificity of the antibody has been confirmed by HCDM Council (HLDA X workshop).

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[12]).

Accuracy

Accuracy of the method was measured on BD FACSCanto™ II flow cytometer and determined as a comparison of the device DryFlowEx TBNK 6-color with similar product available on the market KOMBITEST TBNK 6-color (Cat. No. ED7733) by parallel staining of 118 healthy blood donors.

On Beckman Coulter DxFLEX and Sysmex XF 1600 flow cytometers, the accuracy of the method was determined by comparing the results of analysing the same blood specimens stained by the device DryFlowEx TBNK 6-color on BC DxFLEX and BD FACSCanto™ II (92 healthy donors) flow cytometers respectively and on Sysmex XF-1600 and BD FACSCanto™ II (71 healthy donors) flow cytometers.

Accuracy of the method has been supported by parallel staining of 46 patients (see Table 7) suspected of having immune system pathological condition.

Linear regression analysis parameters are provided in Tables 4 – 7.

Table 4 Linear regression analysis for lymphocyte subsets in healthy blood donors (comparison of the device DryFlowEx TBNK 6-color with IVD product KOMBITEST TBNK 6-color (EXBIO, Cat. No. ED7733))

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	118	0.9850	+0.0054	0.9696	58.50 - 88.20
	cells/µl	118	0.9993	-9.0904	0.9958	739 - 2492
CD3+CD8+	%	118	1.0618	-0.006	0.9858	6.40 - 47.90
	cells/µl	118	1.0497	-6.1323	0.9910	132 - 947
CD3+CD4+	%	118	1.0067	-0.0015	0.9786	26.00 - 60.60
	cells/µl	118	1.0191	-12.603	0.9936	410 - 1717
CD3-CD16+CD56+	%	118	0.9956	-0.0023	0.9851	4.64 – 33.80
	cells/µl	118	0.9943	-3.9727	0.9848	89 - 593
CD3-CD19+	%	118	0.9885	-0.0015	0.9794	2.60 - 22.70
	cells/µl	118	0.9790	-0.9509	0.9880	56 - 608

n = number of blood samples

Table 5 Linear regression analysis for lymphocyte subsets in healthy blood donors (comparison of ED7736 on BD FACSCanto™ II with ED7736 on BC DxFLEX)

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	92	0.9839	0.0106	0.9823	55.00 – 84.90
	cells/µl	92	0.9992	-0.0833	0.9985	408 - 2525
CD3+CD8+	%	92	1.0187	-0.0051	0.9864	6.25 – 45.40
	cells/µl	92	1.0083	-5.1608	0.9930	130 - 1182
CD3+CD4+	%	92	0.9872	0.0017	0.9935	12.10 – 63.10
	cells/µl	92	0.9869	3.4994	0.9975	108 - 1739
CD3-CD16+CD56+	%	92	0.9857	0.0022	0.9904	4.96 – 32.70
	cells/µl	92	0.9784	5.7585	0.9921	96 - 676
CD3-CD19+	%	92	0.9992	-0.0002	0.9900	3.23 – 21.60
	cells/µl	92	1.0031	-1.0160	0.9916	58 - 418

n = number of blood samples

Table 6 Linear regression analysis for lymphocyte subsets in healthy blood donors (comparison of ED7736 on BD FACSCanto™ II with ED7736 on Sysmex XF-1600)

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	71	0.9942	0.0051	0.9853	53.40 – 85.30
	cells/µl	71	1.0101	-10.313	0.9990	396 - 2440
CD3+CD8+	%	71	0.9718	0.00006	0.9878	11.30 – 47.90
	cells/µl	71	0.9646	2.6825	0.9938	121 - 1111
CD3+CD4+	%	71	0.9885	0.0077	0.9955	13.50 – 63.50
	cells/µl	71	1.0178	-7.2726	0.9972	114 - 1452
CD3-CD16+CD56+	%	71	0.9905	0.0033	0.9915	5.71 – 33.60
	cells/µl	71	0.9789	8.4040	0.9951	102 - 676
CD3-CD19+	%	71	0.9149	0.0009	0.9652	5.11 – 19.20
	cells/µl	71	0.9128	1.8844	0.9780	53 - 386

n = number of blood samples

Table 7 Linear regression analysis for lymphocyte subsets in patients suspected of having immune system pathological conditions (comparison of the device DryFlowEx TBNK 6-color with AQUIOS CL Flow Cytometry System - Beckman Coulter, Inc.

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	46	1.0027	-0.6999	0.98	55.1 – 87.6
	cells/µl	46	0.9805	17.223	1.00	620 - 2710
CD3+CD8+	%	46	1.0033	0.7307	0.94	8.1 – 39.8
	cells/µl	46	1.0595	4.148	0.97	84 - 1130
CD3+CD4+	%	46	1.018	-0.9716	0.97	24.4 – 68.2
	cells/µl	46	0.9884	6.4727	0.99	494 - 1619
CD3-CD16+CD56+	%	46	1.0469	-0.5117	0.98	3.3 – 35.2
	cells/µl	46	1.0543	-11.577	0.99	74 - 1070
CD3-CD19+	%	46	1.0213	0.1708	0.96	4.3 – 33.6
	cells/µl	46	1.036	-0.1163	0.98	62 - 958

n = number of blood samples

Linearity

The linearity of the method was verified on 10 serial dilutions of a leukocyte-enriched blood sample (buffy coat). Cell samples were stained with DryFlowEx TBNK 6-color in hexaplicates. Samples were analyzed using BD FACSCanto™ II, Beckman Coulter DxFLEX and Sysmex XF-1600 flow cytometers. Measured data for the indicated lymphocyte subsets were observed to be linear across the lymphocyte range 87-7031 lymphocytes/µl using BD FACSCanto™ II, 85-6698 cells/µl using Beckman Coulter DxFLEX and 175 - 14799 lymphocytes/µl using Sysmex XF-1600. Cell subsets were in the ranges found in Tables 8 - 10.

Table 8 Linear ranges of lymphocyte subsets analysed by BD FACSCanto™ II

BD FACSCanto™ II	
Lymphocyte Subset	Range (cells/µl)
CD3+	79 - 6427
CD3+CD8+	16 - 1271
CD3+CD4+	57 - 4749
CD3-CD16+CD56+	15 - 1198
CD3-CD19+	8 - 722

Table 9 Linear ranges of lymphocyte subsets analysed by Beckman Coulter DxFLEX

Beckman Coulter DxFLEX	
Lymphocyte Subset	Range (cells/µl)
CD3+	79 - 6251
CD3+CD8+	16 - 1274
CD3+CD4+	57 - 4583
CD3-CD16+CD56+	15 - 1276
CD3-CD19+	8 - 704

Table 10 Linear ranges of lymphocyte subsets analysed by Sysmex XF-1600

Sysmex XF-1600	
Lymphocyte Subset	Range (cells/µl)
CD3+	128 – 10391
CD3+CD8+	53 - 4117
CD3+CD4+	67 - 5421
CD3-CD16+CD56+	32 - 2681
CD3-CD19+	14 - 1090

Limit of detection / Limit of quantification / Assay Cut-off

Linearity data were used to state limit of detection (LOD) and limit of quantitation (LOQ).
Limit of detection has been stated as the lowest non-zero absolute cell count value plus 3×SD (standard deviation) for each lymphocyte subset (see Tables 11 – 13).
Limit of quantitation has been stated as the lowest value in linearity range of analyte concentrations presented as lymphocyte subset absolute count at which the CV from the hexaplicates did not exceed 10% and recovery was in range of 90% - 110% (see Tables 11 – 13).
The assay results are not uniquely diagnostic for a single clinical entity, thus the assay cut-off cannot be estimated.

Table 11 Limits of detection and quantification on BD FACSCanto™ II

BD FACSCanto™ II				
Lymphocyte Subset	**Lowest non-zero cell count (cells/µl)**	**3×SD (SD)**	**LOD (cells/µl)**	**LOQ (cells/µl)**
CD3+	1	0.6 (0.2)	1.6	3
CD3+CD8+	1	0.6 (0.2)	1.6	2
CD3+CD4+	1	0.6 (0.2)	1.6	2
CD3-CD16+CD56+	2	0.9 (0.3)	2.9	5
CD3-CD19+	1	0.3 (0.1)	1.3	8

Table 12 Limits of detection and quantification on Beckman Coulter DxFLEX

Beckman Coulter DxFLEX				
Lymphocyte Subset	**Lowest non-zero cell count (cells/µl)**	**3×SD (SD)**	**LOD (cells/µl)**	**LOQ (cells/µl)**
CD3+	1	0.3 (0.1)	1.3	3
CD3+CD8+	1	0.3 (0.1)	1.3	2
CD3+CD4+	1	0.6 (0.2)	1.6	2
CD3-CD16+CD56+	1	0.3 (0.1)	1.3	2
CD3-CD19+	1	0.6 (0.2)	1.6	8

Table 13 Limits of detection and quantification on Sysmex XF-1600

Sysmex XF-1600				
Lymphocyte Subset	**Lowest non-zero cell count (cells/µl)**	**3×SD (SD)**	**LOD (cells/µl)**	**LOQ (cells/µl)**
CD3+	2	0.3 (0.1)	2.3	2
CD3+CD8+	1	0.6 (0.2)	1.6	2
CD3+CD4+	1	0.6 (0.2)	1.6	8
CD3-CD16+CD56+	1	0.6 (0.2)	1.6	11
CD3-CD19+	1	0.3 (0.1)	1.3	14

Repeatability

The repeatability of the assay was measured on ten blood samples in hexaplicates. Samples were analyzed using BD FACSCanto™ II, Beckman Coulter DxFLEX and Sysmex XF-1600 flow cytometers. Coefficients of variation (CV) are provided in the tables below (Tables 14 - 16).

Table 14 Repeatability of the device on BD FACSCanto™ II

BD FACSCanto™ II					
Lymphocyte Subset	Unit	n	Average	SD	%CV
CD3+	%	10	72.15	0.27	0.38
	cells/µl	10	1454	4.9	
CD3+CD8+	%	10	21.05	0.24	1.18
	cells/µl	10	434	4.8	
CD3+CD4+	%	10	46.68	0.28	0.61
	cells/µl	10	932	5.1	
CD3-CD16+CD56+	%	10	15.38	0.19	1.28
	cells/µl	10	294	3.6	
CD3-CD19+	%	10	11.45	0.15	1.34
	cells/µl	10	217	2.7	

Table 15 Repeatability of the device on Beckman Coulter DxFLEX

Beckman Coulter DxFLEX					
Lymphocyte Subset	Unit	n	Average	SD	%CV
CD3+	%	10	70.90	0,34	0.48
	cells/µl	10	1429	6.3	
CD3+CD8+	%	10	20.33	0.33	1.33
	cells/µl	10	418	5.3	
CD3+CD4+	%	10	45.60	0.27	0.72
	cells/µl	10	911	6.3	
CD3-CD16+CD56+	%	10	16.13	0.25	1.61
	cells/µl	10	308	5.0	
CD3-CD19+	%	10	11.24	0.18	1.69
	cells/µl	10	213	3.3	

Table 16 Repeatability of the device on Sysmex XF-1600

Beckman Coulter DxFLEX					
Lymphocyte Subset	Unit	n	Average	SD	%CV
CD3+	%	10	65.29	1.23	2.22
	cells/µl	10	1090	20.6	
CD3+CD8+	%	10	22.34	0.41	2.30
	cells/µl	10	377	6.90	
CD3+CD4+	%	10	38.12	0.98	2.77
	cells/µl	10	633	16.30	
CD3-CD16+CD56+	%	10	20.92	0.78	3.12
	cells/µl	10	354	13.10	
CD3-CD19+	%	10	11.96	0.44	3.81
	cells/µl	10	193	7.10	

Reproducibility

The reproducibility of the assay on BD FACSCanto™ II and Beckman Coulter DxFLEX was measured on 2 stabilized blood samples (CD-Chex Plus® and CD-Chex Plus® CD4 Low from STRECK). The reproducibility of the assay on Sysmex XF-1600 was measured on 4 stabilized blood samples (CD-Chex Plus® and CD-Chex Plus® CD4 Low and IMMUNO-TROL Low Cells and IMMUNO-TROL Cells from Beckman Coulter in addition). Samples were measured under the same conditions for 15 days using 3 lots of the Device (5 days each). Coefficients of variation (CV) are given in the tables below (Table 17 - 19).

Table 17 Reproducibility of the device on BD FACSCanto™ II

Lymphocyte Subset	Material	Unit	Average	SD	%CV
CD3+	CD-Chex Plus®	%	82.07	0.40	0.49
		cells/µl	1659	8.1	0.49
	CD-Chex Plus® CD4 Low	%	67.87	0.60	0.89
		cells/µl	917	8.1	0.89
CD3+CD8+	CD-Chex Plus®	%	25.67	0.43	1.66
		cells/µl	519	8.6	1.66
	CD-Chex Plus® CD4 Low	%	47.23	0.80	1.69
		cells/µl	638	10.8	1.69
CD3+CD4+	CD-Chex Plus®	%	47.20	0.51	1.08
		cells/µl	954	10.3	1.08
	CD-Chex Plus® CD4 Low	%	9.56	0.18	1.85
		cells/µl	129	2.4	1.85
CD3-CD16+CD56+	CD-Chex Plus®	%	9.51	0.27	2.87
		cells/µl	192	5.5	2.87
	CD-Chex Plus® CD4 Low	%	17.00	0.49	2.89
		cells/µl	230	6.6	2.89
CD3-CD19+	CD-Chex Plus®	%	7.89	0.17	2.18
		cells/µl	158	3.4	2.18
	CD-Chex Plus® CD4 Low	%	14.10	0.18	1.29
		cells/µl	190	2.5	1.29

Table 18 Reproducibility of the device on Beckman Coulter DxFLEX

Lymphocyte Subset	Material	Unit	Average	SD	% CV
CD3+	CD-Chex Plus®	%	81.58	0.35	0.43
		cells/µl	1649	7.2	0.43
	CD-Chex Plus® CD4 Low	%	67.57	0.32	0.48
		cells/µl	913	4.3	0.48
CD3+CD8+	CD-Chex Plus®	%	26.57	0.31	1.17
		cells/µl	537	6.3	1.17
	CD-Chex Plus® CD4 Low	%	48.73	0.41	0.85
		cells/µl	658	5.6	0.85
CD3+CD4+	CD-Chex Plus®	%	45.43	0.53	1.17
		cells/µl	918	10.8	1.17
	CD-Chex Plus® CD4 Low	%	9.17	0.25	2.73
		cells/µl	124	3.4	2.73
CD3-CD16+ CD56+	CD-Chex Plus®	%	9.77	0.15	1.56
		cells/µl	197	3.1	1.56
	CD-Chex Plus® CD4 Low	%	17.21	0.23	1.35
		cells/µl	232	3.1	1.35
CD3-CD19+	CD-Chex Plus®	%	7.99	0.33	4.10
		cells/µl	161	6.6	4.10
	CD-Chex Plus® CD4 Low	%	14.18	0.23	1.63
		cells/µl	192	3.1	1.63

Table 19 Reproducibility of the device on Sysmex XF-1600

Lymphocyte Subset	material	Unit	Average	SD	CV (%)
CD3+	CD-Chex Plus®	%	80.58	0.41	0.51
		cells/μl	1689	8.5	
	CD-Chex Plus® CD4 Low	%	64.19	0.71	1.10
		cells/μl	866	9.6	
	IMMUNO-TROL Cells	%	73.47	0.39	0.53
		cells/μl	930	4.9	
	IMMUNO-TROL Low Cells	%	56.03	0.71	1.26
		cells/μl	431	5.4	
CD3+CD8+	CD-Chex Plus®	%	23.43	0.60	2.54
		cells/μl	490	12.5	
	CD-Chex Plus® CD4 Low	%	43.78	0.99	2.26
		cells/μl	591	13.3	
	IMMUNO-TROL Cells	%	24.11	0.26	1.08
		cells/μl	305	3.3	
	IMMUNO-TROL Low Cells	%	34.74	1.00	2.87
		cells/μl	267	7.7	
CD3+CD4+	CD-Chex Plus®	%	51.31	0.74	1.45
		cells/μl	1073	15.6	
	CD-Chex Plus® CD4 Low	%	12.14	0.84	6.92
		cells/μl	164	11.3	
	IMMUNO-TROL Cells	%	45.17	0.51	1.14
		cells/μl	572	6.5	
	IMMUNO-TROL Low Cells	%	15.83	0.36	2.28
		cells/μl	122	2.8	
CD3-CD16+ CD56+	CD-Chex Plus®	%	8.52	0.28	3.31
		cells/μl	178	5.9	
	CD-Chex Plus® CD4 Low	%	15.53	0.48	3.06
		cells/μl	209	6.4	
	IMMUNO-TROL Cells	%	10.03	0.28	2.80
		cells/μl	127	3.6	
	IMMUNO-TROL Low Cells	%	21.59	0.59	2.74
		cells/μl	166	4.6	
CD3-CD19+	CD-Chex Plus®	%	9.93	0.25	2.48
		cells/μl	208	5.1	
	CD-Chex Plus® CD4 Low	%	18.70	0.31	1.63
		cells/μl	252	4.1	
	IMMUNO-TROL Cells	%	13.03	0.34	2.58
		cells/μl	165	4.3	
	IMMUNO-TROL Low Cells	%	17.45	0.55	3.14
		cells/μl	134	4.2	

12. Clinical performance

Patients suspected of having immune system pathological conditions

Clinical data was collected at a clinical site on 46 patients with suspected immune system pathological conditions. Clinical performance was determined as a comparison of the device DryFlowEx TBNK 6-color with accredited clinical laboratory method (AQUIOS CL Flow Cytometry System - Beckman Coulter, Inc.).

Patient immune status assessment results were evaluated in regard to the immune deficiency (Table 20).

Table 20 Clinical performance of the device DryFlowEx TBNK 6-color

		Immune status assessed by accredited clinical laboratory method	
		Immune system pathological conditions	**Normal condition**
Immune status assessed by the device ED7736 DryFlowEx TBNK 6-color	**Immune system pathological conditions**	9 patients	0 patients
	Normal condition	0 patients	37 patients

13. Expected values

Reference Interval

Laboratories must establish their own normal reference intervals for the lymphocyte subsets identified using DryFlowEx TBNK 6-color from the local population of normal donors due to value variations related to age, gender, clinical characteristics, and ethnicity.

14. Limitations

The device DryFlowEx TBNK 6-color has not been validated for use in specimens collected with heparin or acid citrate dextrose (ACD) anticoagulants in determining relative and absolute counts.

The device DryFlowEx TBNK 6-color is not intended for screening and/or phenotyping of leukemia and lymphoma samples.

Absolute counts are not comparable between laboratories using different equipment from various manufacturers.

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16. Trademarks

BD FACSCanto™ II, BD FACSLyric™, BD Multitest™ and FlowJo™ are registered trademarks of Becton, Dickinson and Company, CD-Chex Plus® is a registered trademark of Streck, Cy™ is registered trademark of Cytiva, VenturiOne® is registered trademark of Applied Cytometry, Infinicyt™ is registered trademark of Cytognos S.L..

17. Revision History

Version 2, ED7736_IFU_v2

1) Updated flow cytometers on which the device has been tested.

2) Updated specimen storage. Endogenous and exogenous interference added.

3) Accuracy updated (BD FACSCanto™ II, Beckman Coulter DxFlex, Sysmex™ XF-1600 flow cytometers)

4) Linearity updated (BD FACSCanto™ II, Beckman Coulter DxFlex, Sysmex™ XF-1600 flow cytometers)

5) Limit of detection / Limit of quantification / Assay Cut-off updated (BD FACSCanto™ II, Beckman Coulter DxFlex, Sysmex™ XF-1600 flow cytometers)

6) Repeatability and reproducibility updated (BD FACSCanto™ II, Beckman Coulter DxFlex, Sysmex™ XF-1600 flow cytometers)

7) Expected values removed

8) References updated

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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.