KOMBITEST B/NK Cell 4-color

50 tests | Cat. No. **ED7735**

IVD CE

Instructions for Use (EN)

Version: ED7735_IFU_v1_EN

Date of Issue: 13-12-2022

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Temperature limit
CE	CE marking of conformity		Keep away from sunlight
	Manufacturer	UK CA	UKCA mark
UDI	Unique Device Identifier		
	Consult instructions for use		
Σ	Contains sufficient for <n> tests		
REF	Catalogue number		
LOT	Batch code		
	Use by date		

1. Intended Purpose

KOMBITEST B/NK Cell 4-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 KOMBITEST B/NK Cell 4-color detects and measures relative percentages and absolute counts of human T cells (CD3+), B cells (CD3-CD19+) and NK cells (CD3-CD16+56+).

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:

- hereditary immunodeficiencies [1, 7]
- autoimmune diseases [2]
- defects in innate immune defense [4, 5]

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 KOMBITEST B/NK Cell 4-color is sufficient for 50 tests and is provided with the following reagent:

1 vial (1 ml) containing a premixed combination of fluorochrome-labeled monoclonal antibodies CD3 FITC / CD16 PE + CD56 PE / CD45 PerCP / CD19 APC, diluted at optimum concentrations in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.

Composition

Table 1 Description of active components

Antigen	Flurochrome	Clone	Isotype	Concentration (µg/ml)
CD3	FITC	TB3	IgG2b	2
CD16	PE	3G8	IgG1	1.5
CD56	PE	LT56	IgG2a	1.5
CD19	APC	LT19	IgG1	2
CD45	PerCP	MEM-28	IgG1	5

5. Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Erythrocyte lysing solution (EXCELLYSE Easy, EXBIO Praha, a.s., Cat. No. ED7066 or CyLyse™ FX, Sysmex Partec GmbH, Cat. No. BD303500)

Deionized water (Reagent-grade)

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

6. Equipment required

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

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

Vortex mixer

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	488	677
APC	630 – 640	660

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

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 reagent 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 venous peripheral blood collected into 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 device KOMBITEST B/NK Cell 4-color alone does not provide enumeration of absolute cell counts.

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

Process the blood specimen no later than 24 hours after collection.

10. Procedure

Preparation of reagent(s) provided

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. Time, for which the reagent is in use (exposed to light and elevated temperature), shall not exceed 4 hours per day.

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.

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.

Quality control

Use Streck CD-Chex Plus® or equivalent control cells 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 cells using KOMBITEST B/NK Cell 4-color reagent 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. For each specimen, label a 12 × 75 mm round bottom test tube with the appropriate sample identification.
2. Pipette 20 µl of KOMBITEST B/NK Cell 4-color reagent into the bottom of the 12 x 75 mm test tube.
3. Pipette 50 µl of well-mixed blood specimen to the bottom of the test 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.
4. Vortex and incubate the test tube for 20 minutes at room temperature in the dark.
5. Add 500 µl of diluted (1X) lysing solution to the test tube.
6. Vortex and incubate the test tube for 10 minutes at room temperature in the dark.

Acquire the stained sample immediately on the 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 KOMBITEST B/NK Cell 4-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.

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.

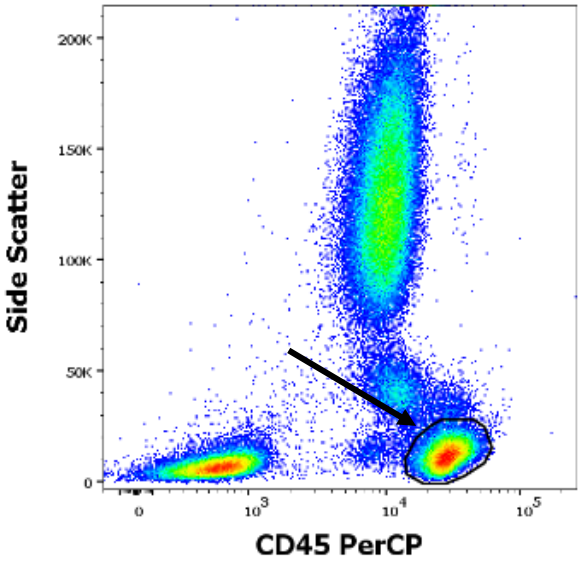
Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

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 KOMBITEST B/NK Cell 4-color stained specimen

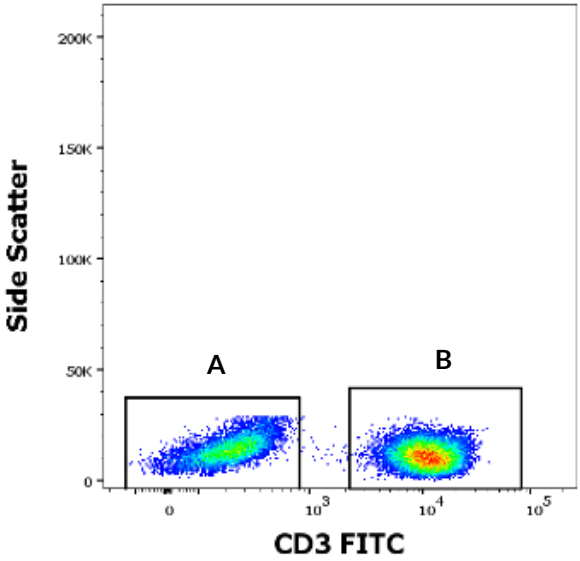
Visualize compensated data in a side-scatter (SSC) versus CD45 PerCP plot. Set the gate for CD45+ lymphocyte population as shown in Figure 1.

Figure 1 Delineation of CD45+ lymphocyte population



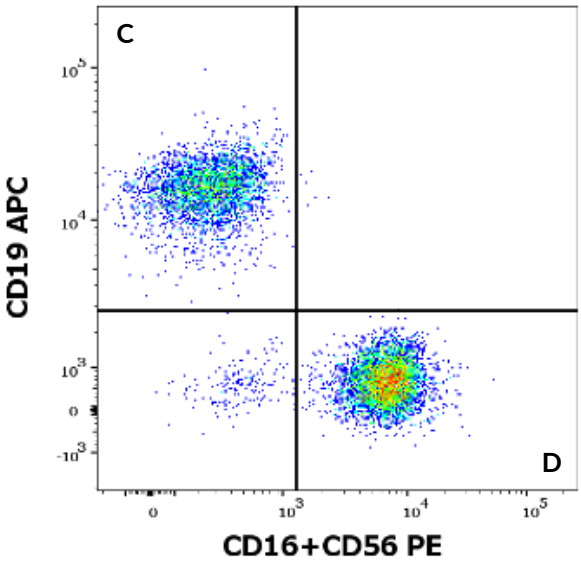
Plot the gated CD45+ lymphocytes in a side-scatter (SSC) versus CD3 FITC plot as shown in Figure 2. Separate CD3+ and CD3- lymphocytes using appropriate gates. Calculate the percentage of T cells (CD3+; region B on the Figure 2) from all lymphocytes.

Figure 2 Separation of CD3+ and CD3- lymphocytes



Plot the gated CD3- lymphocytes (region A on the Figure 2) as CD19 APC versus CD16+CD56 PE as shown in Figure 3. Set appropriate gates and calculate the percentage of B cells (CD16-CD56-CD19+; region C on the Figure 3) and natural killer (NK) cells (CD16+CD56+CD19-; region D on the Figure 3) from all lymphocytes.

Figure 3 CD3- lymphocytes in a dot-plot CD19 APC vs. CD16+CD56 PE



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

NOTICE: All analytical performance data were measured using erythrocyte lysing solution EXCELLYSE Easy (EXBIO Praha, a.s., Cat. No. ED7066).

Specificity

The antibody TB3 recognizes human CD3 antigen of the TCR/CD3 complex. Specificity of the antibody has been confirmed by HCDM Council (HLDA XI workshop).

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 [6]).

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 [3]).

Accuracy

Accuracy of the method was determined as a comparison of the device KOMBITEST B/NK Cell 4-color with similar products available on the market or with other well-documented methods by parallel staining of 30 healthy donors and 81 patients suspected of having immune system pathological condition. Linear regression analysis parameters are provided in Table 3 and 4.

Table 3 Linear regression analysis for lymphocyte subsets in healthy donors (comparison of the device KOMBITEST B/NK Cell 4-color with IVD product BD Multitest™ CD3/CD16+CD56/CD45/CD19 (Cat. No. 342416))

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	30	1.01	-0.010	1.00	50.47 – 85.47
	cells/µl	30	1.00	-0.721	1.00	627 - 2184
CD3-CD16+CD56+	%	30	1.01	-0.001	1.00	5.29 – 35.77
	cells/µl	30	1.01	-0.805	1.00	85 - 992
CD3-CD19+	%	30	0.99	0.002	1.00	5.19 – 26.10
	cells/µl	30	0.99	3.190	0.99	71 - 331

n = number of blood samples

Table 4 Linear regression analysis for lymphocyte subsets in patients suspected of having immune system pathological conditions (comparison of the device KOMBITEST B/NK Cell 4-color with AQUIOS CL Flow Cytometry System - Beckman Coulter, Inc. and a cocktail of single color conjugated antibodies from different manufacturers and analysis on the BD FACSCanto™ II)

Lymphocyte Subset	Unit	n	Slope	Intercept	R^2	Range
CD3+	%	81	1.042	-2.976	0.97	23.4 – 93.6
	cells/µl	81	1.005	-0.010	1.00	140 - 5178
CD3-CD16+CD56+	%	81	1.061	-0.626	0.98	1.6 – 66.7
	cells/µl	81	1.078	-0.017	0.99	10 - 2555
CD3-CD19+	%	81	1.023	-0.163	0.99	0.0 – 69.7
	cells/µl	81	1.032	-0.006	1.00	0 - 4586

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 KOMBITEST B/NK Cell 4-color in hexaplicates. Samples were analyzed using BD FACSCanto™ II flow cytometer and Beckman Coulter DxFLEX flow cytometer. Measured data for the indicated lymphocyte subsets were observed to be linear across the lymphocyte range 368 - 10634 cells/µl using BD FACSCanto™ II and 328 - 9061 cells/µl using Beckman Coulter DxFLEX. Cell subsets were in the ranges found in Tables 5 and 6.

Table 5 Linear ranges of lymphocyte subsets analysed by BD FACSCanto™ II

BD FACSCanto™ II	
Lymphocyte Subset	Range (cells/µl)
CD3+	227 - 6163
CD3-CD16+CD56+	59 - 1609
CD3-CD19+	34 - 912

Table 6 Linear ranges of lymphocyte subsets analysed by Beckman Coulter DxFLEX

Beckman Coulter DxFLEX	
Lymphocyte Subset	Range (cells/µl)
CD3+	217 - 6051
CD3-CD16+CD56+	69 - 1669
CD3-CD19+	33 - 889

Repeatability

The repeatability of the assay was measured on ten blood samples in hexaplicates. Samples were analyzed using BD FACSCanto™ II flow cytometer and Beckman Coulter DxFLEX flow cytometer. Coefficients of variation (CV) are provided in the tables below (Table 7 and 8).

Table 7 Repeatability of the device on BD FACSCanto™ II

BD FACSCanto™ II					
Lymphocyte Subset	Unit	n	Average	SD	%CV
CD3+	%	10	66.47	0.29	0.44
	cells/µl	10	1362	6.19	0.44
CD3-CD16+CD56+	%	10	18.66	0.21	1.26
	cells/µl	10	374	4.36	1.26
CD3-CD19+	%	10	13.69	0.20	1.57
	cells/µl	10	284	4.35	1.57

Table 8 Repeatability of the device on Beckman Coulter DxFLEX

Beckman Coulter DxFLEX					
Lymphocyte Subset	Unit	n	Average	SD	%CV
CD3+	%	10	65.99	0.59	0.92
	cells/µl	10	1352	11.67	0.92
CD3-CD16+CD56+	%	10	19.08	0.44	2.44
	cells/µl	10	382	8.62	2.44
CD3-CD19+	%	10	13.55	0.34	2.59
	cells/µl	10	281	6.73	2.59

Reproducibility

The reproducibility of the assay was measured on 2 stabilized blood samples (CD-Chex Plus® and CD-Chex Plus® CD4 Low) under the same conditions for 15 days using 3 lots of the Device (5 days each). Samples were analyzed using BD FACSCanto™ II flow cytometer and Beckman Coulter DxFLEX flow cytometer. Coefficients of variation (CV) are given in the tables below (Table 9 and 10).

Table 9 Reproducibility of the device on BD FACSCanto™ II

Lymphocyte Subset	Material	Unit	Average	SD	%CV
CD3+	CD-Chex Plus®	%	77.39	0.24	0.31
		cells/µl	1909	5.97	0.31
	CD-Chex Plus® CD4 Low	%	61.38	0.55	0.90
		cells/µl	891	8.04	0.90
CD3-CD16+CD56+	CD-Chex Plus®	%	10.57	0.19	1.84
		cells/µl	261	4.81	1.84
	CD-Chex Plus® CD4 Low	%	19.28	0.46	2.37
		cells/µl	280	6.64	2.37
CD3-CD19+	CD-Chex Plus®	%	11.20	0.13	1.13
		cells/µl	276	3.12	1.13
	CD-Chex Plus® CD4 Low	%	17.95	0.38	2.13
		cells/µl	261	5.55	2.13

Table 10 Reproducibility of the device on Beckman Coulter DxFLEX

Lymphocyte Subset	Material	Unit	Average	SD	%CV
CD3+	CD-Chex Plus®	%	76.77	0.27	0.36
		cells/µl	1894	6.77	0.36
	CD-Chex Plus® CD4 Low	%	60.53	0.38	0.62
		cells/µl	878	5.45	0.62
CD3-CD16+ CD56+	CD-Chex Plus®	%	10.83	0.21	1.96
		cells/µl	267	5.23	1.96
	CD-Chex Plus® CD4 Low	%	19.54	0.31	1.61
		cells/µl	284	4.55	1.61
CD3-CD19+	CD-Chex Plus®	%	11.36	0.23	2.03
		cells/µl	280	5.68	2.03
	CD-Chex Plus® CD4 Low	%	18.23	0.43	2.38
		cells/µl	265	6.31	2.38

12. Clinical performance

Patients with primary immunodeficiency

Clinical data was collected at a clinical site on 30 patients with suspected Common Variable Immune Deficiency (CVID). Clinical performance of the device ED7735 was determined as a comparison of the device KOMBITEST B/NK Cell 4-color using with erythrocyte lysing solution EXCELLYSE Easy (EXBIO Praha, a.s., Cat. No. ED7066) 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 11).

Table 11 Clinical performance of the device KOMBITEST B/NK Cell 4-color – CVID patients

		Immune status assessed by accredited clinical laboratory method	
		Immune deficiency	**Normal condition**
Immune status assessed by the device ED7735 KOMBITEST B/NK Cell 4-color	**Immune deficiency**	23 patients	0 patients
	Normal condition	0 patients	7 patients

13. Expected values

Reference Interval

Reference intervals for the device KOMBITEST B/NK Cell 4-color were determined in a cohort of subjects using erythrocyte lysing solution EXCELLYSE Easy (EXBIO Praha, a.s., Cat. No. ED7066) and the BD FACSCanto™ flow cytometer. Subjects were healthy normal adults (blood donors).

Table 12 Representative reference intervals for the KOMBITEST B/NK Cell 4-color

LymphocyteSubset	Unit	n	Mean	95% Range
CD3+	%	30	69.33	49.24 – 89.43
	cells/µl	30	1293	524 – 2062
CD3-CD16+CD56+	%	30	18.18	0.23 – 36.12
	cells/µl	30	349	0 – 802
CD3-CD19+	%	30	11.75	2.26 – 21.23
	cells/µl	30	209	80 - 228

CAUTION: Indicated values using the device are intended to be representative only. Each laboratory must establish its own reference intervals from the local population of normal donors.

14. Interfering substances and limitations

The device KOMBITEST B/NK Cell 4-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 KOMBITEST B/NK Cell 4-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.

15. References

1) Boldt, A et al. Eight-color immunophenotyping of T-, B-, and NK-cell subpopulations for characterization of chronic immunodeficiencies Cytometry B Clin Cytom. 2014 May;86(3):191-206. doi: 10.1002/cyto.b.21162.
2) Kucuksezer, U C et al. The Role of Natural Killer Cells in Autoimmune Diseases. Front Immunol. 2021 Feb 25;12:622306. doi: 10.3389/fimmu.2021.622306.
3) McMichael AJ, ed. Leucocyte Typing III: 54 White Cell Differentiation Antigens. New York, NY: Oxford University Press; 1987.
4) Orange, J S. Natural killer cell deficiency. J Allergy Clin Immunol. 2013 Sep;132(3):515-525. doi: 10.1016/j.jaci.2013.07.020.
5) Orange, J S. How I Manage Natural Killer Cell Deficiency. J Clin Immunol. 2020 Jan;40(1):13-23. doi: 10.1007/s10875-019-00711-7.
6) Schlossman SF, Boumsell L, Gilks W, et al, eds.: Leucocyte Typing V: White Cell Differentiation Antigens. New York, NY: Oxford University Press; 1995.
7) van Dongen, J J M et al. EuroFlow-Based Flowcytometric Diagnostic Screening and Classification of Primary Immunodeficiencies of the Lymphoid System. Front Immunol. 2019 Jun 13;10:1271. doi: 10.3389/fimmu.2019.01271.

16. Trademarks

17. Revision History

Version 1, ED7735_IFU_v1
Initial release

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.