BasoFlowEx Kit

100 tests | Cat. No. **ED7043**

IVD CE 2265

Instructions for Use (EN)

Version: ED7043_IFU_v9_EN

Date of Issue: 13-02-2026

Symbols used in the device labeling

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

1. Intended Purpose

BasoFlowEx Kit is intended for the determination of basophil activation in peripheral whole blood by flow cytometry.

What is detected and/or measured

The device BasoFlowEx Kit detects and enumerates percentage of activated basophils (CD63+) from all basophil granulocytes (CD203c+ SSC low).

Device function

The device is aid to diagnosis of IgE-mediated allergy if used in conjunction with specific allergen stimulation and relevant clinical information.

Context of a physiological or pathological state

Type I (IgE-mediated) substance hypersensitivity reaction in patients suffering allergy if used in combination with allergen.

Type of assay

Not automated

Quantitative

Type of specimen required

Human heparin and EDTA anticoagulated whole blood.

Testing population

For use in patients suspected of having allergies, particularly in cases where traditional diagnostic methods, like skin prick tests or serum-specific IgE tests, may be inconclusive, impractical, or may possess high risk to patient (e.g. allergen provocation test).

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 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 provisions, where applicable.

3. Test principle

The principle of the test is based on the detection of basophil surface marker CD203c and the activation marker CD63, which is expressed on degranulated basophils. The presence of a stimulation control or allergen is required to induce basophil activation in vitro and subsequent degranulation [(1)]. A positive stimulation control is a sample in which basophils are activated by an anti-IgE monoclonal antibody and the chemotactic peptide N-formyl-Met-Leu-Phe (fMLP), which activates basophils via the fMLP receptor (FPR1). If sufficient allergen-specific IgE molecules are present in the sample, which are bound to the surface of basophils via the high-affinity receptor FcεRI, the added positive stimulus control or allergen causes receptor bridging, which leads to full basophil activation associated with the process of shedding the contents of inflammatory mediators from the cytoplasmic granules to the outside cells (degranulation). Degranulation results, among other things, in the exposure of the cytoplasmic granule transmembrane protein CD63 to the surface of basophils [(2)]. The latter is subsequently detected by an anti-CD63 monoclonal antibody (clone MEM-259) conjugated with FITC. The basophil population is identified in the leukocyte mixture by anti-CD203c monoclonal antibody (clone NP4D6) conjugated to PE.

4. Reagent(s) provided

Contents

The device BasoFlowEx Kit is sufficient for 100 tests and is provided with the following reagents:

Stimulation Buffer (5 lyophilized vials) 1 vial is sufficient for stimulation of 20 blood samples (ED7043-1).

Stimulation Control (2 lyophilized vials) 1 vial is sufficient for stimulation of 25 positive controls (ED7043-2).

Staining reagent (1 vial) 2 ml of premixed antibody cocktail: anti-CD63, FITC labeled and anti-CD203c, PE labeled (ED7043-3).

Lysing Solution (1 bottle) 30 ml of ready to use solution with a fixative (ED7043-4).

Composition

Table 1 Description and concentrations of active components

Antigen	Flurochrome	Clone	Isotype	Concentration (µg/ml)
CD63	FITC	MEM-259	IgG1	50
CD203c	PE	NP4D6	IgG1	13

5. Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

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

Allergens

6. Equipment required

Automatic pipette with disposable tips (10 – 1000 µl) for pipetting specimen and reagents

Liquid dispenser or pipette with disposable tips (5 ml) for dispensing deionized water

Vortex mixer

Thermostat (air incubator) or water bath able to incubate test tubes at 37 °C

Flow cytometer laser excitation source (488 nm), detectors for scatters, optical filters and emission detector appropriate to collect signal from fluorochrome provided in Table 2.

Table 2 Spectral characteristic of fluorochrome used in the device

Fluorochrome	Excitation [nm]	Emission [nm]
PE	488	576
FITC	488	525

NOTICE: The device was tested on flow cytometers BD FACSCanto™ II (BD Biosciences) and DxFLEX (Beckman Coulter).

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

WARNING: Lysing Solution (ED7043-4) contains formaldehyde (CAS No. 50-00-0) and methanol (CAS No. 67-56-1) in concentrations classified as hazardous.

Label elements	Signal word
 	Danger
H-phrases	H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H331: Toxic if inhaled. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. EUH071: Corrosive to the respiratory tract.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P280: Wear protective gloves/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P403+P233: Store in a well-ventilated place. Keep container tightly closed.

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 lyophilized reagents provided is a white powder (Stimulation Buffer and Stimulation Control). Do not use the reagent if you observe any change in appearance, for example a color change or liquefaction.

Normal appearance of the Lysing Solution 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 in specimen receptacle classified as a medical device, with the presence of heparin anticoagulant or EDTA.

Follow the instructions of the tube manufacturer to ensure that the correct filling volume is achieved.

Blood specimen in the collection tube with heparin must be stored at room temperature and must be treated within 48 hours after collection.

Blood specimen in the collection tube with EDTA must be stored in the refrigerator and must be treated within 48 hours after collection.

Samples stained according standard protocol are fixed. Fixed samples may be stored at 2-8 °C for one day in the dark.

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
Triglycerides	Elevated triglyceride levels corresponded to higher monocytes and basophil counts.	4
Erythrocytes	Insufficient lysis, red blood cells present in sample may affect cell counting.	5
Hemoglobin	Hemolyzed samples may produce unreliable results.	6
Bilirubin	Bilirubin may increases	7

	fluorescence background of cells due to its high autofluorescence.	
Lipemia	High circulating levels of lipids may affect flow cytometry analysis of certain blood cell populations.	8

Exogenous Interference

Specimen older than 48 hours may yield erroneous results.

Refrigerated specimen may yield erroneous results.

Aeroallergens (pollen, mites, dust) may contaminate open tubes in laboratory and generate increased basophil activation in negative control which may be higher than cut-off and may decrease assay sensitivity. Blood samples and stimulation tubes must be covered by lids[3].

10. Procedure

Preparation of reagent(s) provided

Stimulation Buffer

Reconstitute lyophilized Stimulation Buffer using 2 ml of demineralized water. Store the unused volume of the buffer at 2-8 °C up to 5 days. Alternatively, the buffer can be aliquoted, frozen once, and stored at ≤ -20 °C for later use.

CAUTION: Avoid repeated freeze/thaw cycles.

Stimulation Control

Reconstitute lyophilized Stimulation Control using 0.25 ml of demineralized water. Store the unused volume of the reagent at 2-8 °C up to 30 days. Alternatively, the reagent can be aliquoted, frozen once, and stored at ≤ -20 °C for later use.

CAUTION: Avoid repeated freeze/thaw cycles.

Staining Reagent

Reagent is ready to use.

Lysing Solution

Reagent is ready to use.

Specimen staining

1. For examination of one patient's specimen, label three 12 x 75 mm round bottom test tubes with the appropriate sample identification and marking for

   negative control reaction,

   positive control reaction,

   and for stimulation with different allergens.

   Pipette to the bottom of the test tubes:

   - **NOTHING** into the tube marked as **negative control reaction**.
   - 10 µl of Stimulation Control into the tube marked as **positive control reaction**.
   - 10 µl of allergen into tube marked as **allergen-stimulated sample** or use a single test tube with lyophilised allergen (EXBIO).

2. Add 100 µl of Stimulation Buffer into all tubes.
3. Pipette 100 µl of heparinized or EDTA whole blood into all tubes and vortex gently.

   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. Place the test tubes into a water bath for 15 minutes or into an air incubator for 25 minutes, both at 37 °C.
5. Add 20 µl of Staining Reagent into all tubes, vortex gently, and incubate for 20 minutes at 2-8 °C or on ice.
6. Add 300 µl of Lysing Solution to each of the test tubes. Vortex gently and incubate the test tubes for 5 minutes at room temperature in the dark.
7. Add 3-4 ml of deionized water into each of the test tubes, vortex gently, and incubate for 5-10 minutes at room temperature in the dark until the red blood cells are lysed.
8. Centrifuge tubes for 5 minutes at 300x g.
9. Remove the supernatant and resuspend the pellet in 0.2-0.4 ml of 1X PBS buffer.
10. Acquire the stained sample immediately on the flow cytometer. If the stained sample will not be acquired immediately, store at 2-8 °C in the dark. Stained samples should be analyzed within 24 hours of receiving the sample.

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 BasoFlowEx Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturer's 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 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.

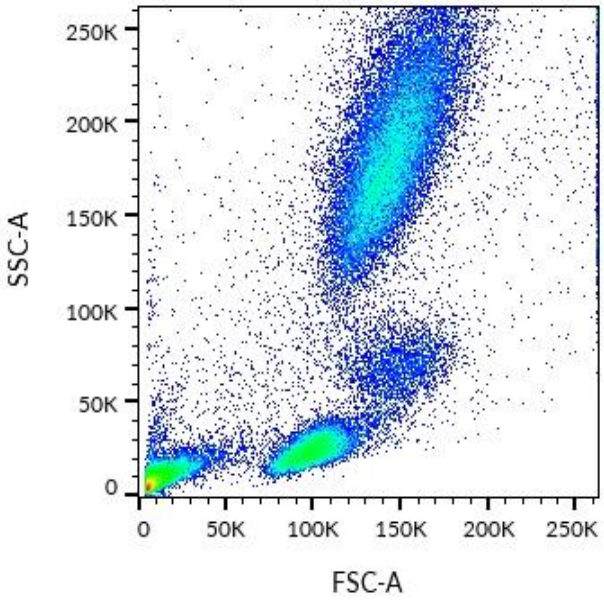
For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

Analysis of a patient's sample

In order to analyze sufficient number of basophils (>200), acquire at least 50,000-100,000 events per sample. Compensate your data (FITC and PE channels) prior to or after the acquisition.

Create dot plot the side-scatter (SSC-A) versus the forward-scatter (FCS-A) as is visualized in Figure 1 to see distribution of leukocytes into lymphocytes, monocytes and granulocytes.

Figure 1 Leukocyte population (FCS-A / SSC-A) (data acquired on BD FACSCanto™ II)



Visualize acquired events in the side-scatter (SSC) versus fluorescence intensity in PE channel (CD203c PE) dot-plot. Set the gate for basophil population (CD203c ^{pos}, SSC ^{low}) as shown in Figure 2. Make sure that both positive and negative control events obtained lie in the gate created, as shown in Figure 2 and Figure 3.

Figure 2 Delineation of basophil population (CD203c^{pos} / SSC^{low}) in positive control sample (data acquired on BD FACSCanto™ II)

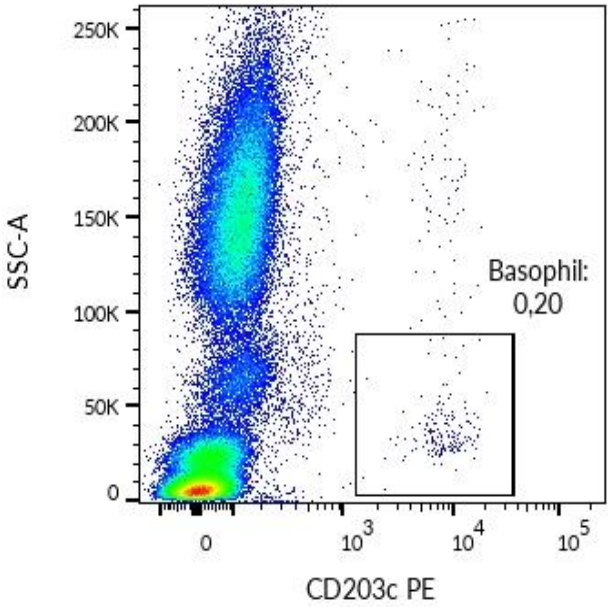
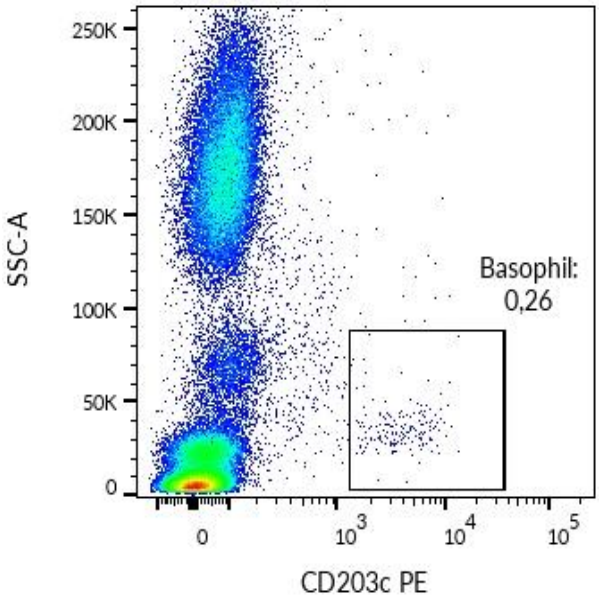


Figure 3 Delineation of basophil population (CD203c^{pos} / SSC^{low}) in negative control sample (data acquired on BD FACSCanto™ II)



Visualize gated basophils as histograms where the X-axis represents fluorescence intensity in FITC (CD63 FITC) channel shown in Figures 4a, b. Use the negative control reaction to set an appropriate gate for non-stimulated basophils (CD63-) and apply it to all samples of the given patient.

Figure 4a Histogram of negative control basophils (data acquired on BD FACSCanto™ II)

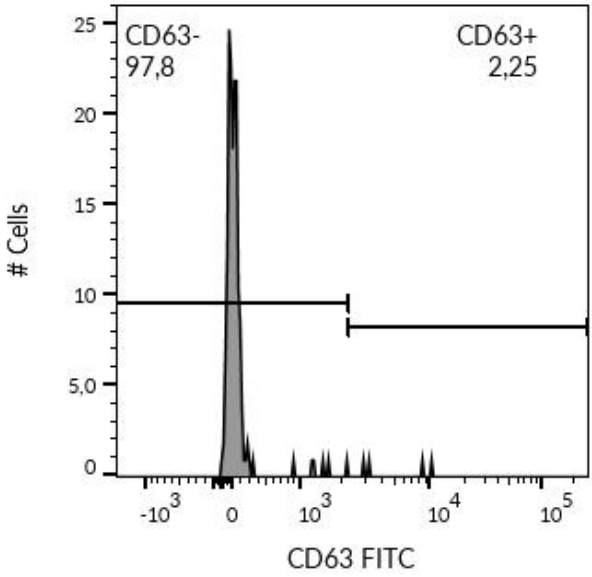
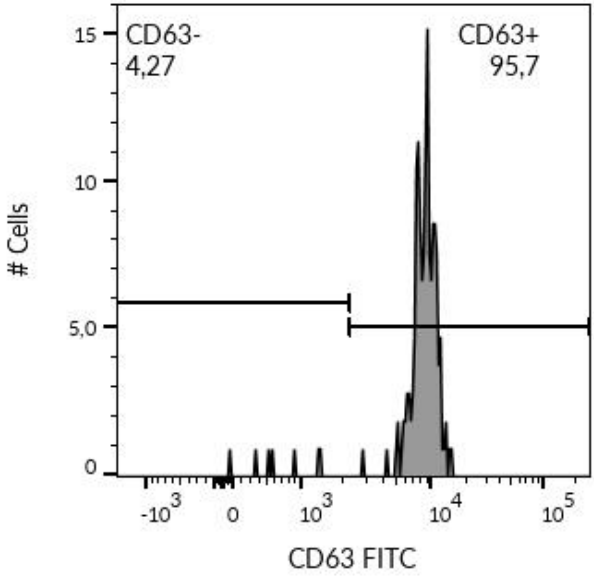


Figure 4b Histogram of positive control basophils
(data acquired on BD FACSCanto™ II)



Calculation and interpretation of analytical results

Analyze the aquired data with appropriate flow cytometry analysis software. Use the negative control reaction to set an appropriate gate for non-stimulated basophils (CD63-) and applied it to all stimulated samples for the same patient. Calculate the percentage of activated basophils ($CD63^{bright}$) in the positive control sample.

If the positive control sample shows activation of basophils < 10 %, the samples cannot be interpreted. The result should be evaluated as invalid and the test should be repeated.

11. Analytical performance

Precision (repeatability and reproducibility)

Repeatability (within-laboratory and within one day and one operator) of the assay were determined from the data measured by activaton of basophils with stimulation control in ten parallels on blood sample with heparin and EDTA under the same experimental conditions. The measuring was performed on two flow cytometers (BD FACSCanto™ II and BC DxFLEX).

Table 4 Repeatability parameters for BasoFlowEx Kit

Type of cytometer	Specimen Type	Mean [%]	Within-lab [%CV]
BD FACSCanto™ II	EDTA	89.8	2.1
	Heparin	82.9	6.2
BC DxFLEX	EDTA	84.4	2.7
	Heparin	80.3	4.9

Reproducibility of the assay were determined from the data measured by activation of basophils with stimulation control by four operators in five parallels on one blood sample with heparin and EDTA under the same experimental conditions. The measuring was performed on two flow cytometers (BD FACSCanto™ II and BC DxFLEX). Furthermore, the intermediate accuracy (interlaboratory, interday) was determined in ten parallels on a blood sample with heparin and EDTA under the same experimental conditions, on two flow cytometers (BD FACSCanto™ II and BC DxFLEX).

Table 5 Reproducibility parameters for BasoFlowEx Kit

Type of cytometer	Specimen Type	Reproducibility CV [%]	Inter-lab [%CV]	Inter-day [%CV]
BD FACSCanto™ II	EDTA	2.4	1.6	1.1
	Heparin	3.3	1.6	4.1
BC DxFLEX	EDTA	5.7	6.5	6.5
	Heparin	4.3	2.9	6.1

Cut-off of activated basophils measured in positive control 60 heparinized blood samples and 102 EDTA blood samples, tested according to standard protocol on two flow cytometers (BD FACSCanto™ II and BC DxFLEX) are provided in Table 6. Based on the results, a technical cut-off of 10 % stimulated basophils has been established. Results above the 10 % threshold are considered positive basophils stimulation.

Table 6 Cut-off value of activated basophils on two specimens and two platforms

Parameter	Cytometer	Blood	n	Mean + 3SD [%]
Activated basophils of negative control	BD FACSCanto™ II	Heparin	60	7.2
	BC DxFLEX	Heparin	60	7.2
	BD FACSCanto™ II	EDTA	102	7.6
	BC DxFLEX	EDTA	102	9.9

Linearity

The linearity of the method was verified on 7 serial dilutions of an EDTA whole blood sample stimulated with ED7043-2 Stimulation Control diluted with an unstimulated EDTA blood sample. Cell samples were stained with BasoFlowEx Kit in tetraplicates. Samples were analyzed using BD FACSCanto™ II and Beckman Coulter DxFLEX flow cytometers. Measured data for the basophil subset was observed to be linear across the range 9.3 – 93.5 % stimulated basophils using BD FACSCanto™ II and 9.1 – 83.7 % stimulated basophils using Beckman Coulter DxFLEX.

12. Clinical performance

Clinical performance of the BasoFlowEx Kit was evaluated by combining scientific peer-reviewed literature and published experience gained from routine diagnostic testing published until September 2024. Although with differing intentions and hypotheses, evaluated publications have investigated and confirmed the ability of the BasoFlowEx Kit to distinguish between allergic and non-allergic patients in the presence of food and pollen allergens, allergens to house pets, and drugs [9, 10, 11]. This capability has also been validated by comparison with other allergen tests such as sIgE and SPT (skin prick test), and with the patient's clinical history and also with other commercially available devices [12]. It has also been confirmed that basophils can be stimulated in a variety of blood samples and the range over which the kit measures are relevant for the diagnosis of allergies [11]. The Basophil Activation Test has been also described as a promising tool for monitoring the efficacy of immunotherapy and in the process of desensitization [12, 13].

13. Expected values

Reference interval

Expected values of activated basophils measured in positive and negative control in 60 heparinized blood samples and 102 EDTA blood samples, tested according to standard protocol on two flow cytometers (BD FACSCanto™ II and BC DxFLEX) are provided in Table 7 and 8.

Table 7 Value of activated basophils of positive control on two specimens and two platforms

Parameter	Cytometer	Blood	n	MIN [%]	MAX [%]
Activated basophils of positive control	BD FACSCanto™ II	Heparin	60	24.2	98.1
	BC DxFLEX	Heparin	60	24.1	90.2
	BD FACSCanto™ II	EDTA	102	21.9	100
	BC DxFLEX	EDTA	102	27.0	96.7

Table 8 Value of activated basophils of negative control on two specimens and two platforms

Parameter	Cytometer	Blood	n	MIN [%]	MAX [%]
Activated basophils of negative control	BD FACSCanto™ II	Heparin	60	0	6.2
	BC DxFLEX	Heparin	60	0	6.4
	BD FACSCanto™ II	EDTA	102	0	11.5
	BC DxFLEX	EDTA	102	0	13.9

14. Limitations

The device BasoFlowEx Kit has not been validated for basophil activation in specimens collected with acid citrate dextrose (ACD) as anticoagulant.

15. References

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13) Ebo, DG et al. Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. Cytometry. Part B, Clinical cytometry 2007; 72(3): 196-203. doi:10.1002/cyto.b.20142.

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

BD FACSCanto™ II and FlowJo™ are registered trademarks of Becton, Dickinson and Company. DxFLEX is registered trademark of Beckman Coulter, Inc.. VenturiOne® is registered trademark of Applied Cytometry. Infinicyt™ is registered trademark of Cytognos S.L..

18. Revision History

Version 9, ED7043_IFU_v9
Change in hazard classification for a component ED7043-4 Lysing Solution.

19. Manufacturer

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20. Authorized Representatives

N/A

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