EXCELLYSE Easy

100 ml | Cat. No. **ED7066**

IVD CE

Instructions for Use (EN)

Version: ED7066_IFU_v7_EN

Date of Issue: 15-02-2023

Symbols used in the device labeling

IVD	In Vitro diagnostic medical device		Keep away from sunlight
CE	CE marking of conformity		Keep Dry Keep away from rain
	Manufacturer	CONTENTS	Contents
UDI	Unique Device Identifier		Caution
	Consult instructions for use	CONC 10×	Concentrated solution (10x)
REF	Catalogue number		
LOT	Batch code		
	Use by date		
	Temperature limit		

1. Intended Purpose

EXCELLYSE Easy is a lysing solution, intended for red blood cell lysis and white blood cell fixation after human peripheral whole blood staining with fluorochrome-conjugated antibodies prior to flow cytometry analysis.

What is detected and/or measured

N/A. Reagent is a lysing solution.

Device function

N/A. Reagent is a lysing solution intended for in vitro diagnostic procedures relating to flow cytometry analysis.

Context of a physiological or pathological state

N/A. Reagent is a lysing solution.

Type of assay

N/A. Reagent is a lysing solution.

Type of specimen required

Human anticoagulated peripheral whole blood specimen.

Testing population

N/A. Reagent is a lysing solution.

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

N/A. Reagent is a lysing solution causing hypotonic lysis of red blood cells while preserving white blood cells for flow cytometry analysis.

4. Reagent(s) provided

Contents

The device EXCELLYSE Easy is sufficient for 2000 reactions of Lyse/no wash procedure or 1000 reactions of Lyse/wash procedure of blood samples lyses and is provided with the following reagent(s):

1 bottle (100 ml) containing 10X concentrated solution.

5. Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

Phosphate buffered saline (1X PBS), pH 7.4 (0.2 g/L KH_2PO_4, 1.42 g/L $Na_2HPO_4 \cdot 2H_2O$, 8.0 g/L NaCl, 0.2 g/L KCl)

Appropriate fluorescent-dye-labeled primary/secondary antibodies

6. Equipment required

Automatic pipette with disposable tips (50 - 1000 µl) for pipetting specimen and reagents

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

Graduated cylinders to measure the volume of deionized water and EXCELLYSE Easy reagent for preparation of the lysing solution in its working concentration (1X)

Centrifuge

Vortex mixer

Flow cytometer

7. Storage and handling

Store at 2-25 °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: EXCELLYSE Easy (ED7066) contains 2,2-oxybisethanol (CAS No. 111-46-6), 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. H335 May cause respiratory irritation. H341 Suspected of causing genetic defects. H350 May cause cancer. H371 May cause damage to organs. H373 May cause damage to the kidneys through prolonged or repeated exposure if swallowed. H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled.
P-phrases	P201 Obtain special instructions before use. P260 Do not breathe vapours. P264 Wash hands and exposed parts of the body thoroughly after handling. P280 Wear protective gloves/eye protection/face protection. P362+P364 Take off contaminated clothing and wash it before reuse. P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302+P352 IF ON SKIN: Wash with plenty of water and soap. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308+P313 IF exposed or concerned: Get medical advice/attention. P314 Get medical advice/attention if you feel unwell. P333+P313 If skin irritation or rash occurs: Get medical advice/attention.

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 EDTA or Heparin anticoagulant.

10. Procedure

Preparation of reagent(s) provided

Bring the reagent to room temperature prior to use.

The reagent is 10X concentrated and must be diluted with deionized water prior use (1 volume of the concentrated solution and 9 volumes of deionized water).

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.

The diluted lysing solution (1X) is stable for 1 month when stored in a liquid dispenser or closed container at room temperature.

Preparation of materials required but not provided

Bring deionized water and 1X PBS to room temperature prior to use.

Quality control

N/A. Reagent is a lysing solution.

Lyse/no wash lysing protocol

1. For each specimen, label (12 × 75 mm) a round bottom test tube with the appropriate sample identification.
2. Follow antibody manufacturer's instructions for whole blood staining.
3. Add 450-1000 µl of diluted lysing solution per 50 µl of whole blood. Mix the content of the tube with a vortex mixer.
4. Incubate for about 5-10 minutes, until the blurry blood sample solution becomes clear.
5. Analyze the processed sample immediately using flow cytometer. If the stained sample will not be acquired immediately, store at 2-8 °C in the dark and analyze within 24 hours.

Lyse/wash lysing protocol

1. For each specimen, label (12 × 75 mm) a round bottom test tube with the appropriate sample identification.
2. Follow instructions for whole blood antibody staining.
3. Add 1000 µl of diluted lysing solution per 50 µl of whole blood. Mix the content of the tube with a vortex mixer.
4. Incubate for about 5-10 minutes, until the blurry blood sample solution becomes clear.
5. Centrifuge the tube for 5 minutes at 300 g.
6. Decant supernatant and resuspend the pellet with 0.2 – 0.5 ml of 1X PBS.
7. Analyze the processed sample immediately using flow cytometer. If the stained sample will not be acquired immediately, store at 2-8 °C in the dark and analyze within 24 hours.

Flow cytometry analysis

The flow cytometer selected for use with the device EXCELLYSE Easy 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™).

Representative data

Figure 1 Two-dimensional density dot-plot showing clusters of peripheral blood leukocytes of EXCELLYSE Easy lyse/no wash processed sample analyzed on BD FACSCanto™ II cytometer.

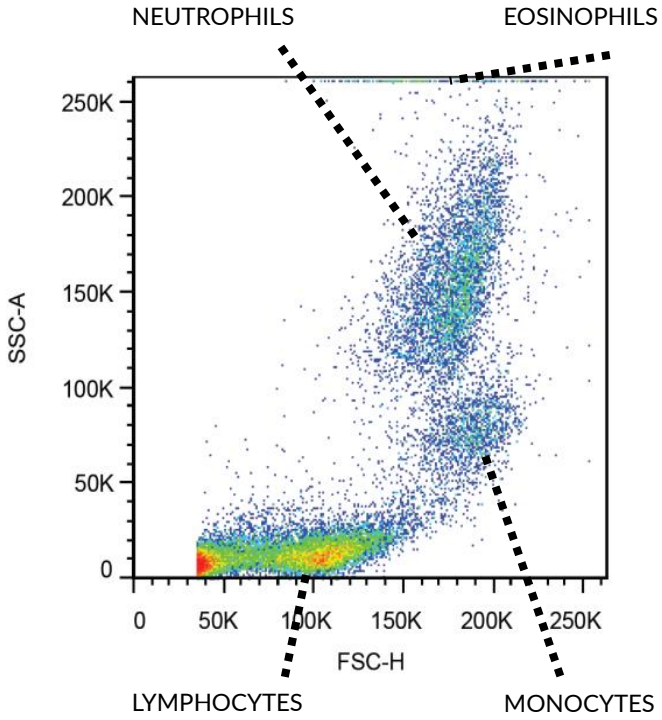


Figure 2 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/no wash protocol and analyzed on BD FACSCanto™ II cytometer.

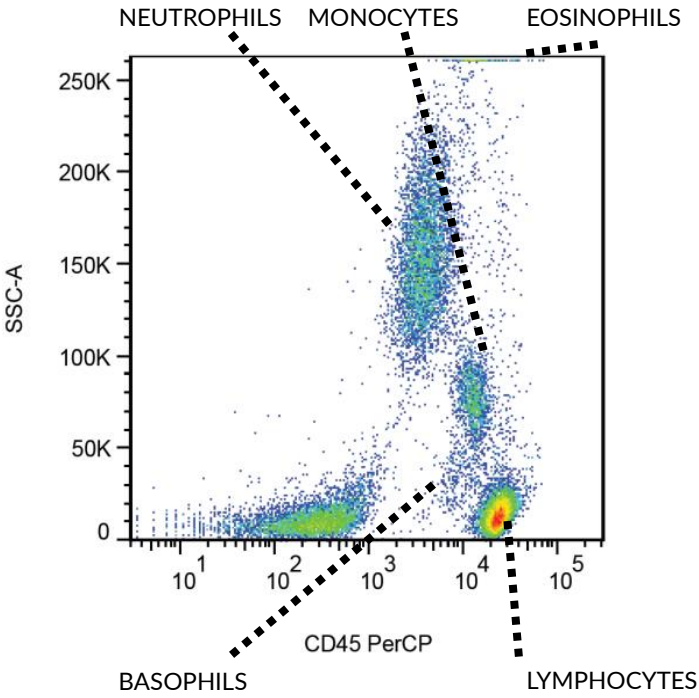


Figure 3 Two-dimensional density dot-plot showing clusters of peripheral blood leukocytes of EXCELLYSE Easy lyse/wash processed sample analyzed on BD FACSCanto™ II cytometer.

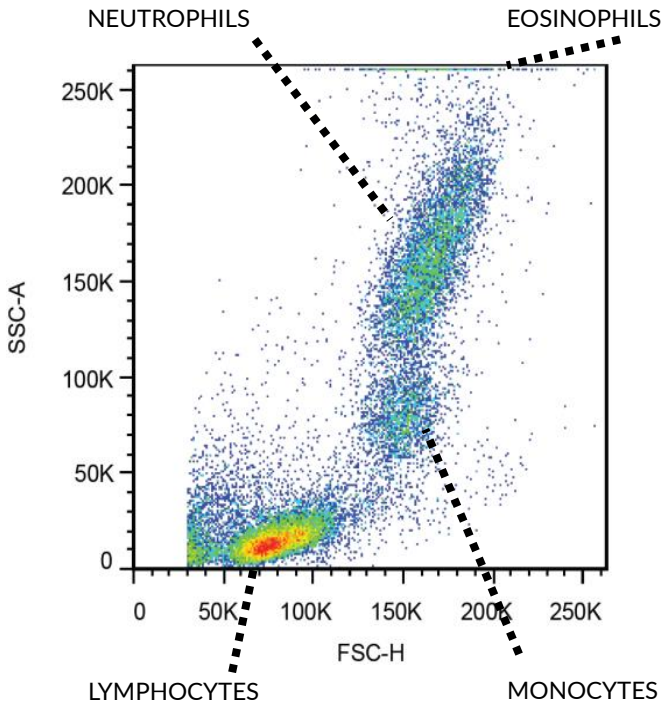
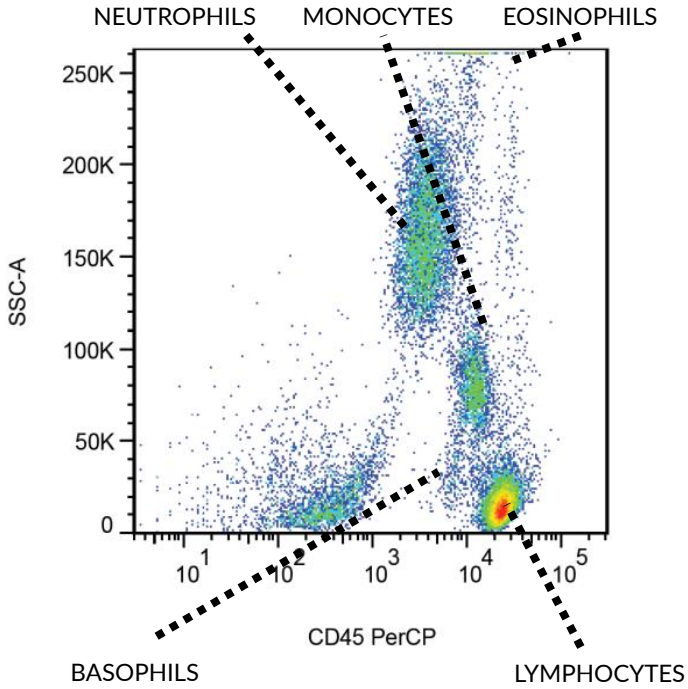


Figure 4 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/wash protocol and analyzed on BD FACSCanto™ II cytometer.



Calculation and interpretation of analytical results

N/A Reagent is a lysing solution.

11. Analytical performance

The device performance was characterized by white blood cell recovery, sample stability in time after its processing and by tube–to-tube variability of T-, B-, NK lymphocyte counts identified and enumerated with CE IVD device ED7735 KOMBITEST B/NK Cell 4-color.

White blood cell recovery

EDTA (n=13) and Heparin (n=5) anticoagulated specimens were processed using EXCELLYSE Easy and analyzed in pre-dilution mode of automated hematology analyzer SYSMEX XN1000. The recovery was determined as the ratio of WBC count in processed sample to WBC count in whole blood before lysis.

Pseudo-processed samples (non-lysed, not centrifuged, diluted with PBS) were analyzed in parallel.

Table 1 WBC recovery in EXCELLYSE Easy processed blood samples

	WBC recovery	
	Mean (%)	**SD (%)**
No wash		
EDTA	94	5
Heparin	95	2
Pseudo-processed	94	3
Wash		
EDTA	93	4
Heparin	94	3
Pseudo-processed	97	2

WBC count stability in time after sample processing

EDTA (n=9) and Heparin (n=5) anticoagulated specimens were processed with EXCELLYSE Easy. The WBC counts were analyzed immediately in pre-dilution mode of automated hematology analyzer SYSMEX XN1000 and again after 24 hours of storage (overnight) in a refrigerator. The change is reported as percent (%) increase or decrease.

Table 2 Change of WBC counts between EXCELLYSE Easy processed samples analysed immediately and after 24 hours of storage

	WBC count relative change	
	Mean (%)	**SD (%)**
No wash		
EDTA	-1	2
Heparin	-1	3
Wash		
EDTA	0	5
Heparin	2	2

T-, B-, NK- lymphocyte count stability in time after sample processing

EDTA (n=1) and Heparin (n=1) anticoagulated sample were stained in hexaplicates using CE IVD device ED7735 KOMBITEST B/NK Cell 4-color and processed with

EXCELLYSE Easy. The flow cytometry analyses of T-, B-, NK-lymphocyte percentages were analyzed immediately in BD FACSCanto™ II flow cytometer and again after 24 hours of storage (overnight) in a refrigerator. The counts and CV (%) are reported for both measurements.

Table 3 T-, B-, NK-lymphocyte percentages in EXCELLYSE Easy processed samples analysed immediately and after 24 hours of storage

	Frequency of lymphocytes (%)					
	CD3		CD16+56		CD19	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
No wash						
Heparin immediate analysis	75.2	0.3	13.3	0.9	8.2	2.2
Heparin after 24 hours	78.2	0.9	12.2	5.7	8.4	3.5
EDTA immediate analysis	85.3	0.6	7.6	1.7	3.5	1.7
EDTA after 24 hours	87.8	0.5	7.0	4.1	3.5	5.0
Wash						
Heparin immediate analysis	75.1	0.4	14.6	0.7	8.5	2.0
Heparin after 24 hours	76.2	0.4	13.9	2.4	8.6	2.4
EDTA immediate analysis	85.5	0.2	9.8	0.6	3.0	4.0
EDTA after 24 hours	85.5	0.2	9.8	0.6	3.0	4.0

Repeatability (tube-to-tube variability)

Repeatability was measured as tube-to-tube variability of T-, B-, NK-lymphocyte relative counts. The lymphocyte subset counts were identified by whole blood staining with 4-color reagent (CE IVD ED7735 KOMBITEST B/NK Cell 4-color). Hexaplicates of EDTA (n=5) and heparin (n=5) anticoagulated specimens were processed with EXCELLYSE Easy wash and no wash protocols and analyzed on Beckmann Coulter DxFLEX and BD FACSCanto™ II cytometers.

Table 4 Tube-to-tube variation of T-, B-, NK- lymphocyte counts in samples processed with EXCELLYSE Easy and analyzed on BD FACSCanto™ II cytometer

BD FACSCanto™ II	Frequency of lymphocytes (%)					
	CD3		CD16+56		CD19	
	Range	CV (%)	Range	CV (%)	Range	CV (%)
No wash						
Heparin	73 - 80	0.6	8 - 19	2.7	7 - 13	2.5
EDTA	58 - 75	0.7	11 - 28	2.2	4 - 19	3.1
Wash						
Heparin	58 – 77	0.5	9 – 31	2.0	4 – 20	1.7
EDTA	77 - 81	0.5	8 - 20	2.2	7 - 14	2.1

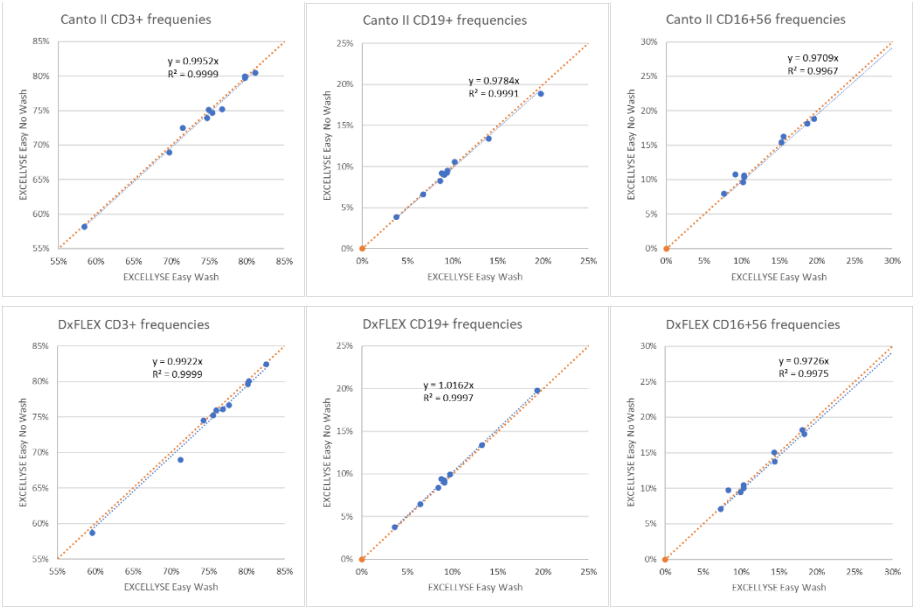
Table 5 Tube-to-tube variation of T-, B-, NK- lymphocyte counts in samples processed with EXCELLYSE Easy and analyzed on Beckmann Coulter DxFLEX cytometer

DxFLEX	Frequency of lymphocytes (%)					
	CD3		CD16+56		CD19	
	Range	CV (%)	Range	CV (%)	Range	CV (%)
No wash						
Heparin	75 - 82	0.6	7 - 18	2.3	6 - 13	3.2
EDTA	59 - 77	0.6	10 - 28	1.5	4 - 20	2.3
Wash						
Heparin	74 - 83	0.3	7 - 18	2.0	6 - 13	2.6
EDTA	60 - 78	0.5	8 - 30	1.7	4 - 19	2.4

Correlation between no wash and wash protocols

Hexaplicates of EDTA (n=5) and heparin (n=5) anticoagulated specimens were stained with 4-color reagent (CE IVD ED7735 KOMBITEST B/NK Cell 4-color) processed with EXCELLYSE Easy wash and no wash protocols. Samples were analyzed immediately on Beckmann Coulter DxFLEX and BD FACSCanto™ II cytometers.

Figure 5 Comparison of T cell, B cell and NK cell relative counts in samples processed with EXCELLYSE Easy wash versus no wash procedure. Samples were analysed in BD FACSCanto™ II (upper row) and Beckmann Coulter DxFLEX (lower row) cytometers.



Reproducibility

N/A.

12. Clinical performance

N/A. Reagent is a lysing solution. It does not yield results that can be correlated with a particular clinical condition or a physiological or pathological process.

13. Expected values

N/A. Reagent is a lysing solution.

14. Limitations

Wash protocol shows lower WBC recovery due to the centrifugation and supernatant decanting.

Use of vacuum aspiration for supernatant removal may cause an unpredictable cell loss and variation in WBC recovery.

15. References

N/A

16. Use of Third Party Trademarks

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

17. Revision History

Version 7, ED7066_IFU_v7

Changing the volume of the diluted lysis solution in step number 3 in section 10. Procedure (Lyse/no wash lysing protocol) from 450 µl to 450-1000 µl. IFU layout changed, texts adapted to comply with IVD regulation. Further details of device performance added.

18. Contact Information

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19. 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.