EXCELLYSE I (RUO)

100 ml | Cat. No. **ED7779**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7779_TDS_v1_EN

Date of Issue: 16-02-2023

Symbols used in the product labeling

RUO	Research Use Only	CONTENTS	Contents
	Manufacturer		
	Consult instructions for use		
REF	Catalogue number		
LOT	Batch code		
	Use by date		
	Temperature limit		
	Keep away from sunlight		
	Keep Dry Keep away from rain		

1. Intended Purpose

EXCELLYSE I 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.

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 product is intended for professional laboratory use only.

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.

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 product EXCELLYSE I is sufficient for 1000 blood samples lyses and is provided with the following reagent(s):

1 bottle (100 ml) containing ready to use solution.

5. Materials required but not provided

12 x 75 mm round bottom test tubes

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 µl – 100 µl) for pipetting specimen and reagents

Liquid dispenser or pipette with disposable tips (1.0 ml – 3.0 ml) for dispensing deionized water

Vortex mixer

Centrifuge

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 I (ED7779) 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	H302 Harmful if swallowed. 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.
P-phrases	P201 Obtain special instructions before use. P264 Wash hands and exposed parts of the body thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection. P301+P312 IF SWALLOWED: Call a 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. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P362+P364 Take off contaminated clothing and wash it before reuse

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.

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.

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 a 12 × 75 mm round bottom test tube with the appropriate sample identification.
2. Follow antibody manufacturer's instructions for whole blood staining.
3. Add 100 µl of lysing solution per 50 µl of whole blood. Mix the content of the tube with a vortex mixer.
4. Incubate for 2-5 minutes at room temperature.
5. Add 1 ml of deionized water to the tube, mix well, and incubate for about 5-10 minutes, until the blurry blood sample solution becomes clear.
6. 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 a 12 × 75 mm round bottom test tube with the appropriate sample identification.
2. Follow antibody manufacturer's instructions for whole blood staining.
3. Add 100 µl of lysing solution per 50 µl of whole blood. Mix the content of the tube with a vortex mixer.
4. Incubate for 2-5 minutes at room temperature.
5. Add 3 ml of deionized water to the tube, mix well, and incubate for about 5-10 minutes, until the blurry blood sample solution becomes clear.
6. Centrifuge the tube for 5 minutes at 300 g.
7. Decant supernatant and resuspend the pellet with 0.2 – 0.5 ml of 1X PBS.
8. 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 product EXCELLYSE I 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 I lyse/no wash processed sample analyzed on BD FACSCanto™ II cytometer.

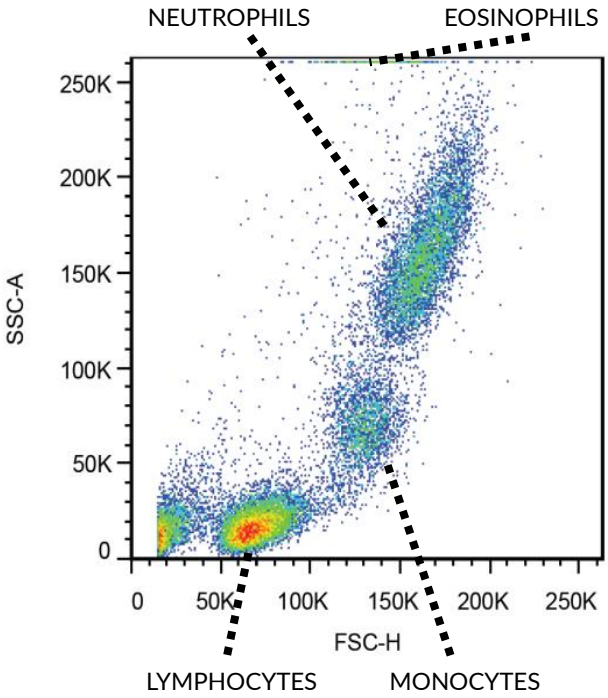


Figure 2 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/no wash protocol and analyzed on BD FACSCanto™ II cytometer.

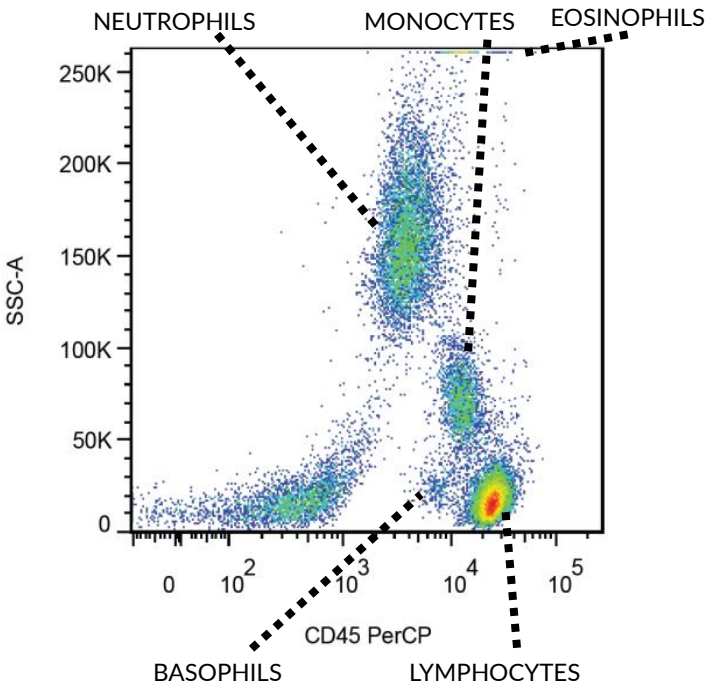


Figure 3 Two-dimensional density dot-plot showing clusters of peripheral blood leukocytes of EXCELLYSE I lyse/ wash processed sample analyzed on BD FACSCanto™ II cytometer.

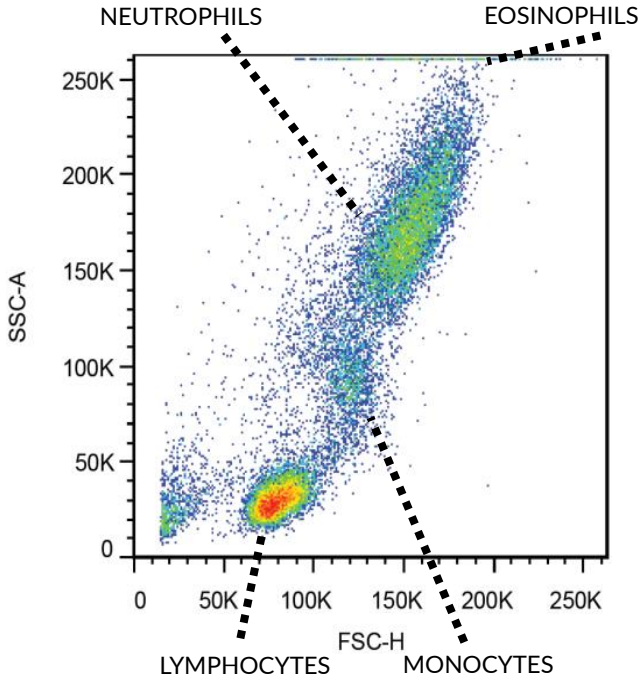
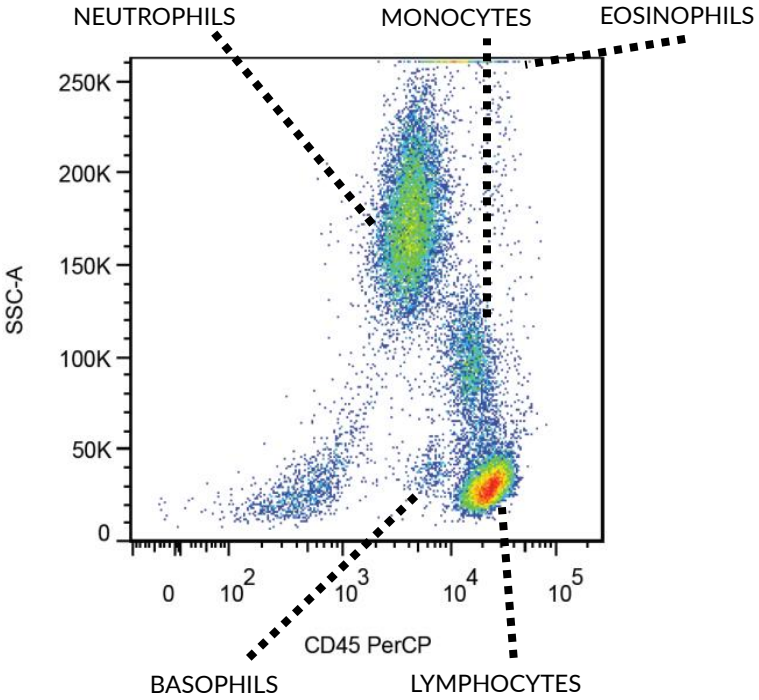


Figure 4 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/wash protocol and analyzed on BD FACSCanto™ II cytometer.



Calculation and interpretation of analytical results

N/A. Reagent is a lysing solution.

11. Analytical performance

The product 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 product ED7735 KOMBITEST B/NK Cell 4-color.

White blood cell recovery

EDTA (n=13) and Heparin (n=5) anticoagulated specimens were processed using EXCELLYSE I 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 I processed blood samples

	WBC recovery	
	Mean (%)	**SD (%)**
No wash		
EDTA	96	6
Heparin	94	9
Pseudo-processed	95	2
Wash		
EDTA	87	5
Heparin	78	6
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 I. 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 I processed samples analysed immediately and after 24 hours of storage

	WBC count relative change	
	Mean (%)	**SD (%)**
No wash		
EDTA	-3	4
Heparin	3	8
Wash		
EDTA	1	2
Heparin	-1	3

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 product ED7735 KOMBITEST B/NK Cell 4-color and processed with

EXCELLYSE I. 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 I 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	75.1	0.3	13.5	1.4	8.3	1.5
Heparin after 24 hours	76.4	0.4	12.9	1.4	8.5	1.5
EDTA immediate analysis	85.8	0.3	8.2	2.1	3.3	2.3
EDTA after 24 hours	86.3	0.6	8.1	3.7	3.3	3.9
Wash						
Heparin immediate analysis	77.1	0.3	13.6	1.3	8.4	1.8
Heparin after 24 hours	78.0	1.2	13.3	4.2	8.1	5.5
EDTA immediate analysis	86.7	0.4	9.1	3.1	2.5	3.2
EDTA after 24 hours	87.7	0.3	8.3	2.5	2.5	5.7

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 (ED7735 KOMBITEST B/NK Cell 4-color). Hexaplicates of EDTA (n=5) and heparin (n=5) anticoagulated specimens were processed with EXCELLYSE I 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 I 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	71 - 81	0.4	8 - 19	2.3	7 - 14	2.0
EDTA	59 - 75	0.7	11 - 29	2.0	4 - 19	2.3
Wash						
Heparin	72 - 81	0.5	8 - 20	2.0	7 - 14	2.2
EDTA	60 - 77	0.6	9 - 29	2.3	4 - 19	3.1

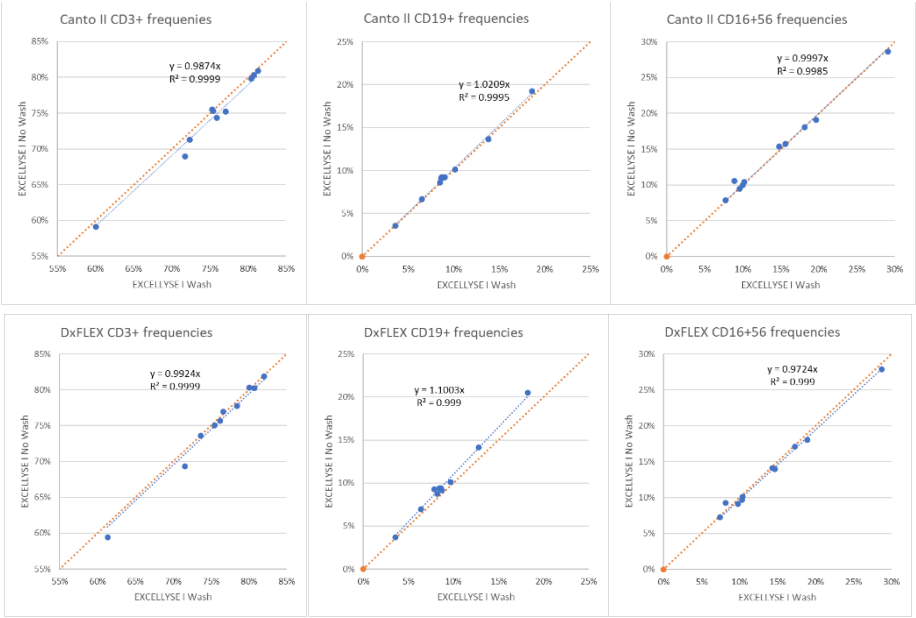
Table 5 Tube-to-tube variation of T-, B-, NK- lymphocyte counts in samples processed with EXCELLYSE I and analyzed on Beckmann Coulter DxFLEX cytometer

DxFLEX	Frequency of lymphocytes (%)					
	CD3		CD16+56		CD19	
	Range	CV (%)	Range	CV (%)	Range	CV (%)
No wash						
Heparin	74 - 82	0.5	7 - 18	3.5	7 - 14	3.1
EDTA	59 - 78	0.6	9 - 28	2.1	4 - 21	3.6
Wash						
Heparin	74 - 82	0.4	7 - 19	1.6	6 - 13	2.6
EDTA	61 - 78	0.6	8 - 29	2.1	4 - 18	2.2

Correlation between no wash and wash protocols

Hexaplicates of EDTA (n=5) and heparin (n=5) anticoagulated specimens were stained with 4-color reagent (ED7735 KOMBITEST B/NK Cell 4-color) processed with EXCELLYSE I 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 I 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. Expected values

N/A. Reagent is a lysing solution.

13. Interfering substances and 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.

14. References

N/A

15. 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..

16. Revision History

Version 1, ED7779_TDS_v1

Initial release

17. Manufacturer

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