EXCELLYSE Live

100 ml | Cat. No. **ED7068**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7068_TDS_v5_EN

Date of Issue: 06-03-2024

Symbols used in the product labeling

RUO	Research Use Only	⚠	Caution
	Manufacturer	CONC 10×	Concentrated solution (10x)
	Consult instructions for use	CONTENTS	Contents
REF	Catalogue number		
LOT	Batch code		
	Use by date		
	Temperature limit		
	Keep away from sunlight		
	Keep Dry Keep away from rain		

Description

The EXCELLYSE Live is a lysing solution for red blood cell lysis following antibody staining of leukocytes in human peripheral whole blood.

Leukocyte analysis and detection in peripheral blood requires elimination of interfering cells, mainly erythrocytes. So far, the Ficoll density gradient method was used to separate leukocytes from whole blood. This method is rather time consuming and may lead to a loss of certain cell subsets. Direct blood sample staining followed by red blood cell lysis therefore takes place in clinical laboratories as a fast and easy method for whole blood flow cytometry analysis.

The EXCELLYSE Live lysing solution allows for red blood cell lysis and is free of fixation component keeping leukocyte membrane intact and the cells viable. This solution is appropriate for use when viable leukocytes are required after red blood cell lysis. The lysing solution is optimized for use with EXBIO single colour monoclonal antibodies and KOMBITEST reagents and may be used in both lyse/wash and lyse/no wash protocol.

Specification

EXCELLYSE Live (10× concentrated solution). Dilute with deionized water prior use (1 volume of concentrated solution and 9 volumes of deionized water). Always prepare freshly diluted 1× solution.

Reagent(s) provided

Contents

The product EXCELLYSE Live is sufficient for 500 blood sample lyses and is provided with the following reagent(s):
1 bottle (100 ml) containing 10× concentrated solution.

Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

Phosphate buffered saline (1× PBS), pH 7.4 (0.2 g/L KH2PO4, 1.42 g/L Na2HPO4·2H2O, 8.0 g/L NaCl, 0.2 g/L KCl)

Appropriate fluorescent-dye-labeled primary/secondary antibodies

Equipment required

Automatic pipette with disposable tips (100 µl – 500 µl) for pipetting specimen and 1× PBS

Liquid dispenser or pipette with disposable tips (1.0 ml – 3.0 ml) for dispensing lysing solution

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

Vortex mixer

Centrifuge

Flow cytometer

Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

Do not freeze.

See Section 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).

Warnings, precautions and limitations of use

GHS Hazard Classification

WARNING: EXCELLYSE Live (ED7068) contains ammonium chloride (CAS No. 12125-02-9) in concentrations classified as hazardous.

H-phrases	H302: Harmful if swallowed. H319: Causes serious eye irritation.

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.

Specimen

Use venous peripheral blood collected into specimen receptacle classified as a medical device, with EDTA or Heparin anticoagulant.

Blood specimen in the collection tube must be stored at room temperature. Do not refrigerate.

Procedure

Preparation of reagent(s) provided

Bring the reagent to room temperature prior to use.

The reagent is 10× concentrated and must be diluted with deionized water prior to 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.

Freshly prepared lysing solution (1×) is stable for 1 day when stored at room temperature.

Preparation of materials required but not provided

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

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 2.0 ml of diluted lysing solution per 100 µl of whole blood. Mix the content of the tube with a vortex mixer.
4. Incubate for about 5-10 minutes at room temperature, 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. See figures 1 and 2 for example data.

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 2.0 ml of diluted lysing solution per 100 µl of whole blood. Mix the content of the tube with a vortex mixer.

4. Incubate for about 5-10 minutes at room temperature, until the blurry blood sample solution becomes clear.
5. Centrifuge the tube for 5 minutes at 300 g.
6. Discard supernatant and resuspend the pellet with 0.2 – 0.5 ml of 1× 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.
8. Fix the cells if samples are required to be stored for more than **2 hours**. Non-fixed cells may change their size. Note that cells lose their viability after fixation. See figures 3 and 4 for example data.

Flow cytometry analysis

The flow cytometer selected for use with the product EXCELLYSE Live 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 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 Live lyse/no wash processed sample analyzed on BD FACSCanto™ II cytometer

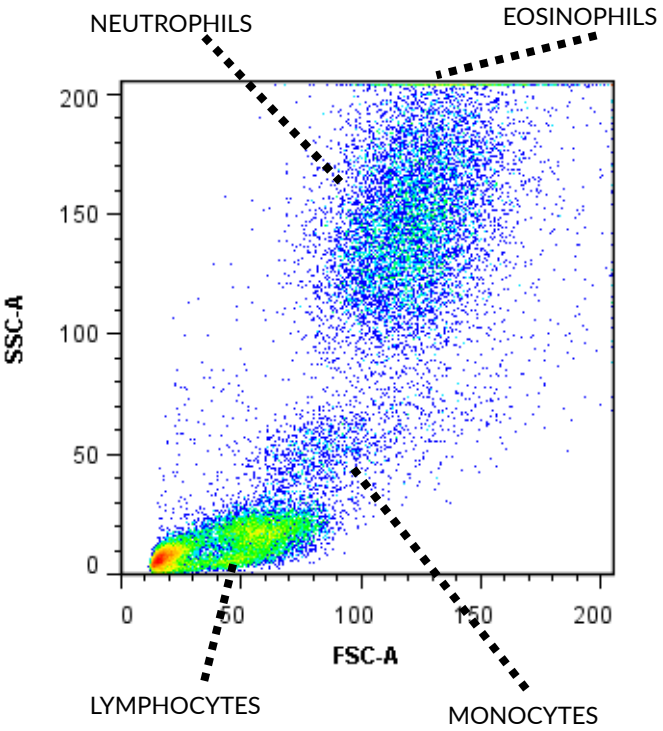


Figure 2 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/no wash protocol and analyzed on BD FACSCanto™ II cytometer

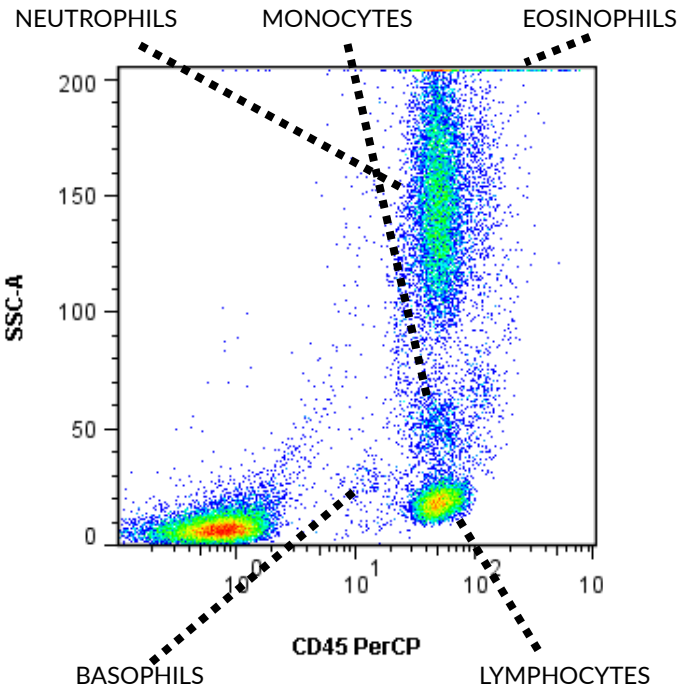


Figure 3 Two-dimensional density dot-plot showing clusters of peripheral blood leukocytes of EXCELLYSE Live lyse/wash processed sample analyzed on BD FACSCanto™ II cytometer

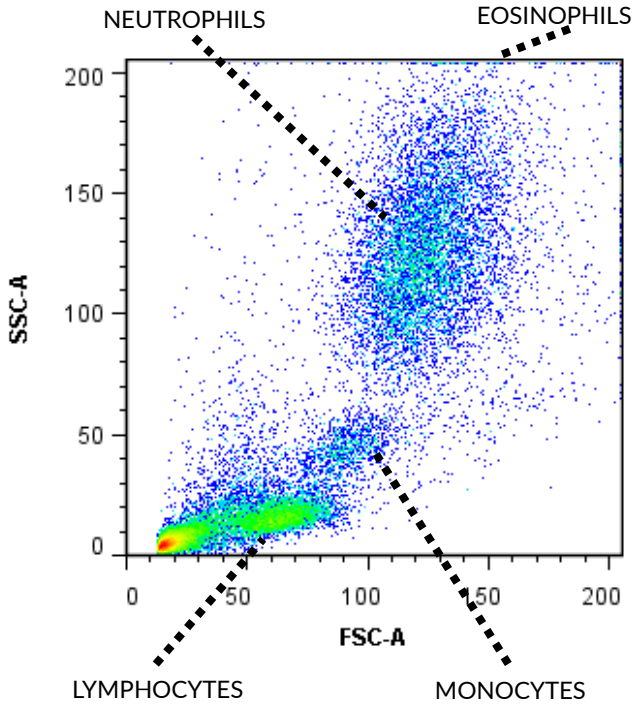
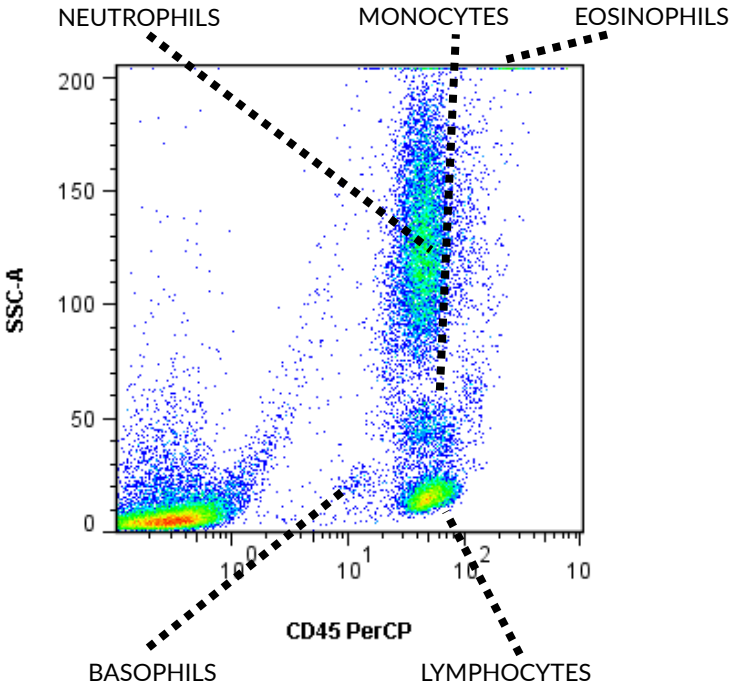


Figure 4 Staining profile of whole blood stained with anti-CD45 PerCP labelled antibody, processed with lyse/wash protocol and analyzed on BD FACSCanto™ II cytometer



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.

References

N/A

Trademarks

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

Revision History

Version 5, ED7068_TDS_v5
TDS layout changed.

Manufacturer

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