EXCELLYSE I

Cat.No. ED7065

1. Intended purpose

Intended use

The EXCELLYSE I lysing solution is intended for red blood cell lysis following antibody staining of human peripheral blood leukocytes.
The lysing solution is appropriate for use with EXBIO single colour monoclonal antibodies and KOMBITEST reagents and may be used in both lyse/wash and lyse/no wash protocol.

Context of a physiological or pathological state

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

2. Test principle

n/a

3. Reagents provided

The content of the vial (100 ml) is sufficient for 500 - 1000 tests. The lysing solution is ready to use.

4. Materials required but not provided

Material necessary for collection of peripheral blood
Suitable 5ml test tubes for blood staining (e.g. 12 × 75 mm)
Phosphate buffered saline (PBS)
Deionized water
Appropriate fluorescent-dye-labeled primary/secondary antibody

5. Equipment required

Automatic pipettes with disposable tips
Vortex mixer
Centrifuge
Flow cytometer

6. Storage and handling

Store the EXCELLYSE I at 2-25 °C.

7. Warnings, precautions and limitations of use

- Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This CE-IVD kit is in conformity with the European Directive 98/79/EC.
- Do not use the reagent after its expiration date.
- Do not use if any discoloration or precipitation occurs.
- Do not freeze.
- Avoid reagents contamination.
- Blood samples are considered as potentially infectious and must be handled with care. Avoid all contact of the sample with the skin, eyes and mucosa.
- The reagent contains formaldehyde and methanol.
  H-phrases
  H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled.
  H317: May cause an allergic skin reaction.
  H351: Suspected of causing cancer.
  P-phrases
  P270: Do not eat, drink or smoke when using this product.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
  P302+P352: IF ON SKIN: Wash with plenty of soap and water.
  P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  P501: Dispose of contents/container to authorized facility for dangerous wastes.
- See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors.
- Any non-performance of lysing protocol may produce false results.

8. Specimen

Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).

9. Procedure

Lyse/no wash lysing protocol

1. Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).
2. Follow instructions for whole blood antibody staining.
3. Add **100 µl of lysing solution** per **50 µl of whole blood**. Mix the tube with a vortex mixer.
4. Incubate for about 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 sample immediately using flow cytometer or store the sample at 2-8°C in the dark and analyze within 24 hours. No further cell fixation is required. See figures 1 and 2 for example data.

Fig. 1: Peripheral blood leukocytes dot-plot from lysed/non-washed whole blood, analyzed on BD FACSCanto™ cytometer.

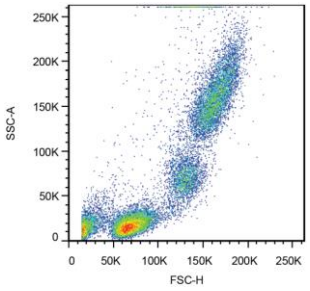
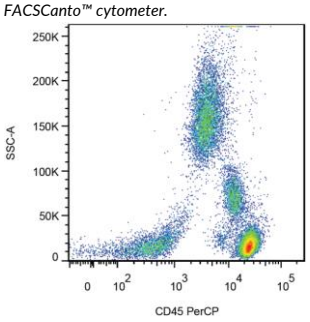


Fig. 2: Identification of CD45+ lymphocyte population in lysed/non-washed whole blood stained with anti-CD45 antibody, analyzed on BD FACSCanto™ cytometer.



Lyse/wash lysing protocol

1. Collect peripheral blood in a sterile tube with an anticoagulant (e.g. Heparin, EDTA).
2. Follow manufacturer's instructions for whole blood antibody staining.
3. Add **100 µl of lysing solution** per **50 µl of whole blood**. Mix the tube with a vortex mixer.
4. Incubate for about 2-5 minutes at room temperature.
5. Add 2-3 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. Remove supernatant and resuspend the pellet with 0.2 – 0.5 ml of PBS.
8. Analyze the sample immediately using flow cytometer or store the sample at 2-8 °C in the dark and analyze within 24 hours. No further cell fixation is required. See figures 3 and 4 for example data.

Fig. 3: Peripheral blood leukocytes dot-plot from lysed/washed whole blood, analyzed on BD FACSCanto™ cytometer.

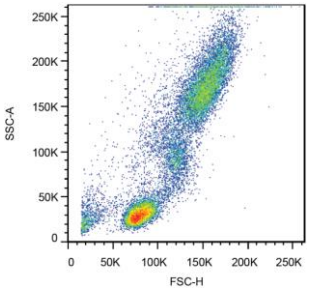
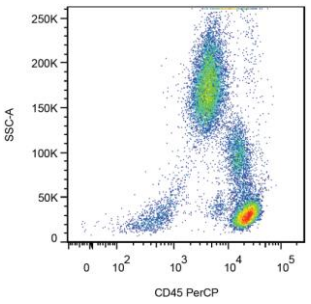


Fig. 4: Identification of CD45+ lymphocyte population in lysed/washed whole blood stained with anti-CD45 antibody, analyzed on BD FACSCanto™ cytometer.



10. Analytical performance

Precision (repeatability and reproducibility)

Repeatability was estimated by enumeration of leukocyte subsets in a single blood sample with KOMBITEST CD3 FITC/CD16+56 PE and KOMBITEST CD3 FITC/ CD19 PE (both EXBIO Praha). Measurements were repeated 10 times during one day using the same blood sample.

Lymphocyte Subset	n	Mean	SD	CV(%)
% $CD3^+$	10	47.4	0.98	2.1
% $CD16^+CD56^+$	10	32.9	0.58	1.8
% $CD19^+$	10	17.3	0.64	3.7

Reproducibility was estimated by enumeration of leukocyte subsets in Immuno-Troll™ Cells (Beckamnn Coulter) with KOMBITEST CD3 FITC/CD16+56 PE and KOMBITEST CD3 FITC/ CD19 PE (both EXBIO Praha). Measurements were performed 14 times during one month using the same LOT of Immuno-Troll™ cells.

Lymphocyte Subset	n	Mean	SD	CV(%)
% $CD3^+$	14	72.8	1.94	2.7
% $CD16^+CD56^+$	14	11.7	0.87	7.4
% $CD19^+$	14	13.2	1.16	8.8

11. Clinical performance

n/a

Interfering substances and limitations

Red blood cells from abnormal patients may be resistant to lysis using lysing solutions.
Flow cytometer may produce false results if the device has not been aligned and maintained appropriately.
Data may be incorrectly interpreted if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.
Blood samples from abnormal patients may exhibit abnormal values of positive cells.

12. References

n/a

13. Manufacturer

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14. Trademarks

n/a

15. Revision History

• Version 1, ED7065_IFU_v1
Initial Release
• Version 2, ED7065_IFU_v2
GHS symbols, H- and P- phrases introduced.
• Version 3, ED7065_IFU_v3
Registered trademark issued to EXCELLYSE ®.
Manufacturer postal code changed from 25242 to 25250.
• Version 4, ED7065_IFU_v4
The smaller version of the product (30 ml vial) is no longer mentioned.
• Version 5, ED7065_IFU_v5
The company logo changed. IFU layout changed. Analytical performance data added. Registered trademark removed from EXCELLYSE.
• Version 6, ED7065_IFU_v6
Product Use Limitation text was refined.

EXCELLYSE I

100 ml | Cat.No. **ED7065**

IVD CE

Instructions for Use

Version ED7065_IFU_v6_EN
Date of Issue: 07-04-2020

EN

Symbols

REF	Catalogue number
LOT	Batch code
	Use-by date
	Temperature limits
IVD	In vitro diagnostic medical device
CE	CE marking of conformity
	Consult instructions for use
	Manufacturer