- Standard Operating Procedure 0008 -

Preparation of Leukocytes by Erythrocyte lysis for Chipcytometry

Short Description

This SOP describes how to isolate leukocytes from whole blood by erythrocyte lysis for biobanking and later analysis by Chipcytometry.

Versioning

Version Number	1.4	valid from: 2019-06-03
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Signatures

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Target Group

	lab personnel Zellkraftwerk; customers Zellkraftwerk
internal use only	NO

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A. Scope

The scope of this standard operating procedure is to describe how to isolate leukocytes from whole blood by erythrocyte lysis for biobanking and later analysis by Chipcytometry.

B. Definitions and Abbreviations

- EDTA: Ethylenediaminetetraacetic acid
- RBC: red blood cell
- RT: room temperature

C. Personnel Qualifications

Basic education and training

- hygiene requirements;
- wearing and use of protective equipment and clothing;
- handling of potentially infectious materials;
- laboratory design, including airflow conditions;
- prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards);
- good laboratory practice;
- organization of workflow;
- waste handling;
- use of equipment (operation, identification of malfunctions, maintenance).

The training shall be:

- given before a staff member takes his/her post;
- strictly supervised;
- adapted to take account of new or changed conditions; and
- repeated periodically, preferably every year.

D. Equipment and Procedure

Principle

Leukocytes are prepared by erythrocyte lysis, resuspended in wash buffer, and then pipetted into ZellSafe chips, using a standard procedure demonstrated in the video available at this website: https://www.youtube.com/watch?v=X1bXN2rbyTU&t. **In case of deviation between this SOP and the video, you must strictly adhere to the steps described in the present document. Avoid pipetting air through the chip channel as soon as a sample is loaded.**

Samples

EDTA - anticoagulated blood. **DO NOT USE HEPARIN-ANTICOAGULATED BLOOD** since erythrocyte lysis is incomplete in heparin-blood using this protocol (for heparin blood a different lysis buffer with adapted osmolarity has to be used).

Equipment and Materials

- pipettes and pipette tips (non sterile)
- appropriate centrifuge
- reaction tubes, e.g. FACS-tubes (BD Falcon, Cat.# 352058)
- ZELLKRAFTWERK washing station (Cat.# 28050606/10-001)
- Light microscope with 20x objective and phase contrast (e.g. Ph1-0.4)

Reagents and Supplies

Component	Storage
Biolegend RBC Lysis buffer (10x; Catalog#420301)	4°C/ 39°F
ZellSafe Rare Chips (Cat.# 28050606/03-010, provided in a ZellSafe box)	4°C/ 39°F
ZellSafe Box (Cat.# 28050606/10-002, for storage of ZellSafe chips)	4°C/ 39°F
ZellScanner ONE Buffer Kit (Cat.# 28050606/07-003) containing: ZELLKRAFTWERK wash buffer ZELLKRAFTWERK fixation buffer ZELLKRAFTWERK storage buffer	 RT RT 4°C/ 39°F

Procedure

A. Erythrocyte lysis

1. Dilute 10x RBC lysis buffer to 1X working concentration with deionized water (e.g. 1800µl deionized water + 200µl 10x RBC lysis buffer).
2. Dilute 100 µl EDTA-anticoagulated blood with 2000 µl Biolegend RBC Lysis buffer (1x) in an appropriate reaction tube.
3. Gently vortex and incubate at room temperature, protected from light, for 15 minutes.
4. Centrifuge the suspension (10 min., 350 g, RT) while keeping the brake ON (acc. 9, dec. 9).
5. Carefully remove and discard the supernatant without touching the pellet. Afterwards, resuspend the pellet in 500 µl ZELLKRAFTWERK wash buffer and centrifuge again (10 min., 350 g, RT; acc. 9, dec. 9).
6. Carefully remove the supernatant completely and resuspend the cell pellet in 40 µl ZELLKRAFTWERK wash buffer.

B. Preparation and loading of the ZellSafe chips

1. Apply the patient identification label on the ZellSafe chip at the position indicated in Fig. 1a (optional; not included in the kit). Please do not write on the barcode label.
2. Place the chip with label side up in the ZELLKRAFTWERK Washing station. Remove the sealing plug from the inlet of the ZellSafe chip (Fig. 1b; do not discard the plugs!). Pipette a few drops of **ZELLKRAFTWERK wash buffer** into the inlet to remove the air.
3. Plug the pipette adapter into the inlet of the ZellSafe chip (Fig. 1c), and fill the adapter with **ZELLKRAFTWERK wash buffer**. Remove any air bubble from the pipette adapter by carefully aspirating the fluid.
4. Remove the sealing plug from the ZellSafe chip outlet. Rinse the chip with 3x 500 µl **ZELLKRAFTWERK wash buffer**. Make sure that all air bubbles are removed and that a flow is established before loading the ZellSafe chip with cell samples. Pipetting of all solutions (buffers and cell suspension) should be done drop-by-drop. The solutions flow through the chip by gravity. **NEVER LET THE CHIPS RUN DRY!**
5. Pipette 40 µl cell solution to the chip and allow the cells to settle (**5 min., RT**).
6. Rinse the chip with 3x 500µl **ZELLKRAFTWERK wash buffer** and verify cell density with a standard light microscope. Please refer to section *"D. Quality control"* on pages 7-8 for examples.
7. Rinse the chip with 3x 500µl **ZELLKRAFTWERK fixation buffer**. Incubate **45 min. at 4°C/ 39°F**.
8. Rinse the chip with 3x 500 µl **ZELLKRAFTWERK wash buffer**.
9. For storage rinse the chip with 3x 500 µl **ZELLKRAFTWERK storage buffer**.
10. Tightly seal the chip with sealing plugs. First seal the outlet, thereafter the inlet.

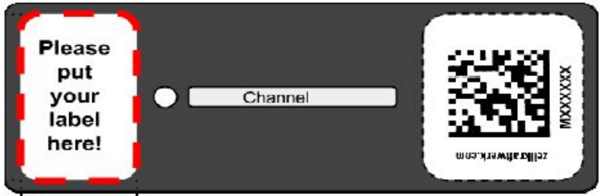

Fig. 1a | Space for additional label on the ZellSafe™ chip (label not included).

Fig. 1b | ZellSafe™ chip with sealing plugs.

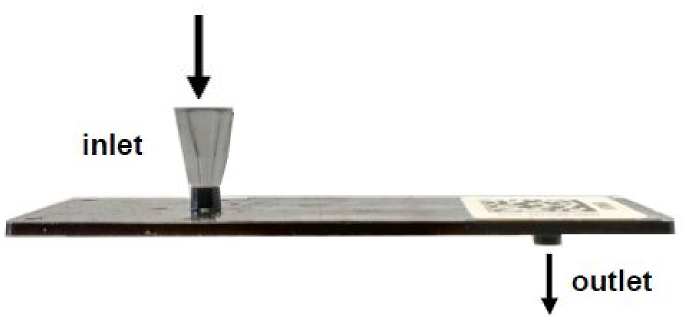

Fig. 1c | Zell Safe ™ chip with pipette adapter.

C. *Quality control - cell density*

After loading cells on the ZellSafe chips, please verify the quality and cell density with a standard light microscope. Examples of acceptable (Fig. 2a) and unacceptable (Fig. 2b) cell densities, and artefacts (Fig. 2c) are given below. If cell densities are too low, consult the “Troubleshooting“ section (page 9).

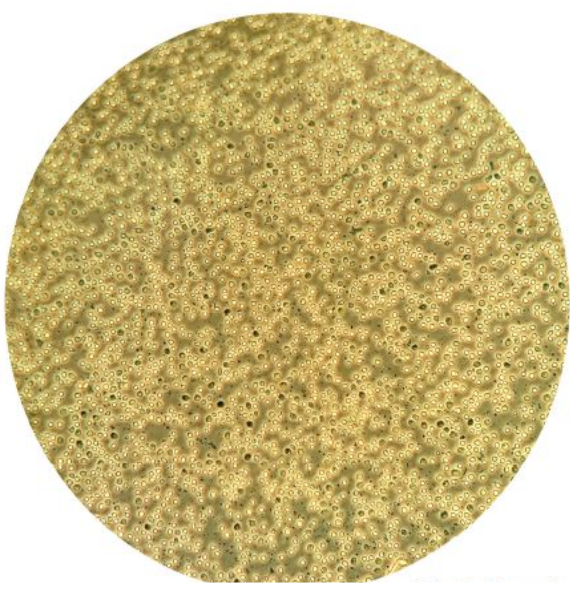

Fig. 2a | Example: acceptable cell density (*200x*)

Fig. 2b | Example: unacceptable cell density (*200x*)

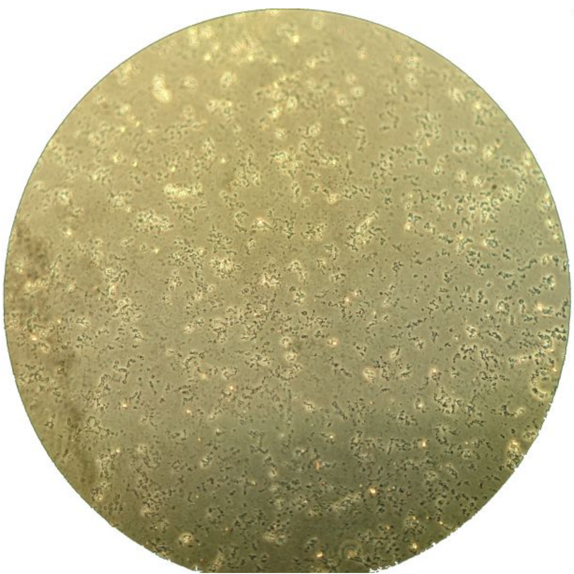

Fig. 2c | Example: Dirt, no cells (*200x*)

Note: The ZellSafe™ chips that are to be shipped should be stored in a ZellSafe™ box.
The shipping conditions are **4°C/ 39.2°F** with temperature tracking (RFID). **DO NOT FREEZE!** Please complete the attached sample manifest and place it to the shipment. Also, please ship the samples as described in detail in the **ZELLKRAFTWERK shipping instructions**.

E. Troubleshooting

Problem	**Possible cause**	**Solution**
Air bubbles in the chip	Air infiltration	Carefully aspirate the fluid or pipette with slight pressure. As soon as cells are loaded pressure should be avoided.
No flow through the chip	Clogged pipette adapter	Replace pipette adapter.
	Trapped air	Carefully aspirate the fluid or pipette with slight pressure. As soon as the cells are loaded, pressure should be avoided.
Low cell count on the chip	Low cell count	If possible, reload the chip with 40 µl of the same sample **BEFORE** fixation.

Appendix 1:
Delivery Data Sheet / Sample Manifest

For ZellSafe chips that are to be shipped, please complete this data sheet and place it to the shipment. The Operator ID can be found on your ZELLKRAFTWERK Certificate.

Chip ID	Sample ID	Sample type, Description of samples	Storage conditions	Operator ID	To be completed by ZELLKRAFTWERK
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
M				**H**	
All items checked for amount and description: Name and Signature (to be signed by Zellkraftwerk)					
All items stored according to storage information: Name and Signature (to be signed by Zellkraftwerk)					

Please provide a phone number and a contact person in case of emergency.

Name: Phone number:

Please note that ZELLKRAFTWERK will not accept the delivery for quality assurance purposes if the delivered items do not match the description.