- Standard Operating Procedure 0002-

Preparation and Biobanking of PBMCs using Ficoll for Chipcytometry

Short Description

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

Versioning

Version Number	1.3	valid from: 2018-11-20
Replaces Version	1.2	from: 2016-04-10

Signatures

Author Nancy Stanslowsky	Date 2018-11-15	Signature 
Reviewer Karen Böttcher	Date 2018-11-20	Signature 
Approval Christian Hennig	Date 2018-11-20	Signature 

Target Group

	lab personnel Zellkraftwerk; customers Zellkraftwerk
internal use only	NO

Table of Content

A. Scope

The scope of this standard operating procedure is to describe how PBMCs are separated from anticoagulated blood by using Ficoll, and how the cells are loaded and fixed on ZellSafe™ chips for subsequent testing.

B. Definitions and Abbreviations

- PBMC: peripheral blood mononuclear cells
- FACS: fluorescence-activated cell sorting
- 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

PBMC are separated from blood by Ficoll gradient centrifugation, 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

Peripheral blood not older than 8 hours and supplemented with anticoagulants (e.g. citrate, heparin, EDTA).
Disclaimer: PBMC quality from older samples may be compromised and clinical data may not be readable.

Equipment and Materials

- Pipettes and pipette tips (non sterile)
- Centrifuge with swinging bucket rotor and tube adapters for 12x75 mm tubes
- 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
Bicoll (Ficoll) (e.g. Biochrom, Cat.# L6115)	RT, in the dark
ZellSafe™ **Cell** Chips (Cat.# 28050606/01-010) or 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

NOTE: 1 ml anticoagulated blood is sufficient to load one ZellSafe™ chip.

A. Ficoll separation

1. Pipette 1.5 ml Ficoll to a FACS-tube.
2. Dilute 1 ml anticoagulated blood with **1 ml ZELLKRAFTWERK wash buffer** and **CAREFULLY** layer the diluted blood over the Ficoll phase. Do not mix.
3. Centrifuge in a swinging-bucket rotor (**10 min; 465 g; RT; Acc. 7; Dec. 1**).
4. After centrifugation, the PBMCs are concentrated in a whitish layer just beneath the plasma layer (Fig. 1). Note: A reddish PBMC layer indicates contamination with red blood cells and reduced sample quality.
5. Carefully collect the PBMC layer with a pipette and transfer this fraction into a new FACS tube.
6. Add **1ml ZELLKRAFTWERK wash buffer**, mix, and centrifuge (**5 min; 100 g; RT**) while keeping the brake ON (Acc. 9 Dec. 9). Do not centrifuge more than 5 min, or at a higher speed, because this causes extensive thrombocyte contamination.
7. Carefully remove and discard the supernatant (containing thrombocytes and debris) without disturbing the pellet. Resuspend the pellet in **1 ml ZELLKRAFTWERK wash buffer** while avoiding the formation of air bubbles, and centrifuge again (**5 min; 100 g; RT**) while keeping the brake ON (Acc. 9, Dec. 9).
8. Carefully remove and discard the entire supernatant without disturbing the pellet. Resuspend the pellet in **100 µl ZELLKRAFTWERK wash buffer**.

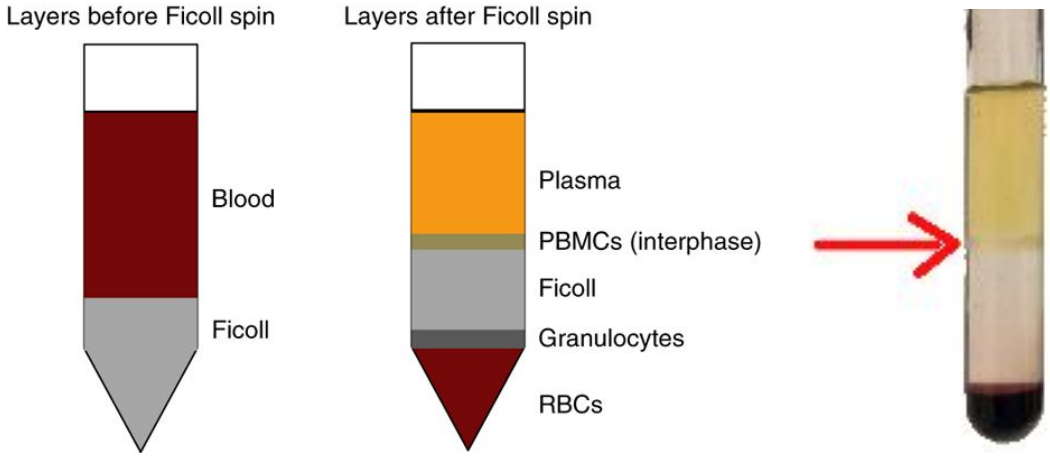

Fig. 1 | Position of the PBMCs after centrifugation (*Source: Lin et al., Nature Protocols 9, 1563-1577 (2014)*)

B. Preparation and loading of the ZellSafe™ chips

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 2a (optional; not included in the kit). Please do not write on the QR-code 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. 2b; 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. 2c), 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 200 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 100 µl cell solution into the chip and allow the cells to settle **(5 min; RT)**.
6. Rinse the chip with 5x 200 µl **ZELLKRAFTWERK wash buffer** and **verify cell density** with a standard light microscope. Please refer to section *"D. Quality control"* on pages 8-9 for examples.
7. Rinse the chip with 5x 200 µl **ZELLKRAFTWERK fixation buffer**. Incubate **45 min at 4°C/ 39°F**.
8. Rinse the chip with 5x 200 µl **ZELLKRAFTWERK wash buffer**.
9. For storage rinse the chip with 5x 200 µl **ZELLKRAFTWERK storage buffer**.
10. Tightly seal the chip with the sealing plugs. First seal the outlet, thereafter the inlet.

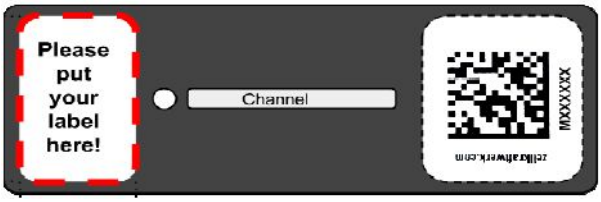

Fig. 2a | Space for additional label on the ZellSafe™ chip (label not included).

Fig. 2b | ZellSafe™ chip with sealing plugs.

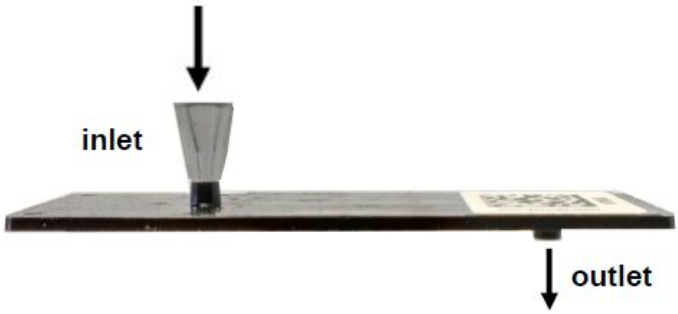

Fig. 2c | Zell Safe ™ chip with pipette adapter.

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**.

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. 3a) and unacceptable (Fig. 3b) cell densities, and artefacts (Fig. 3c) are given below. If cell densities are too low, consult the “Troubleshooting“ section (page 10).

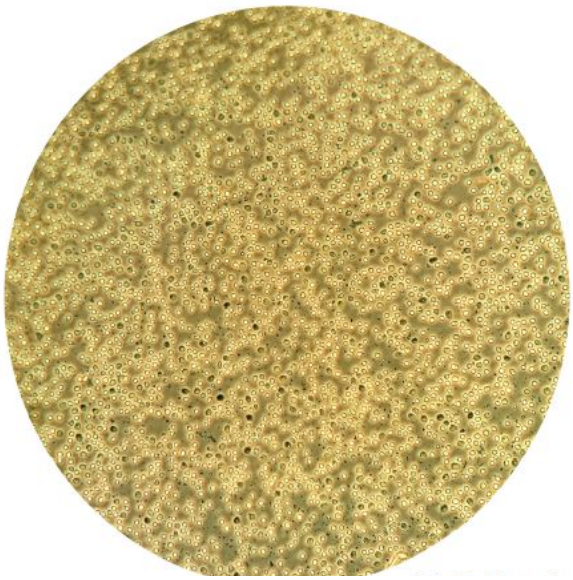

Fig. 3a | Example: acceptable cell density (*200x*)

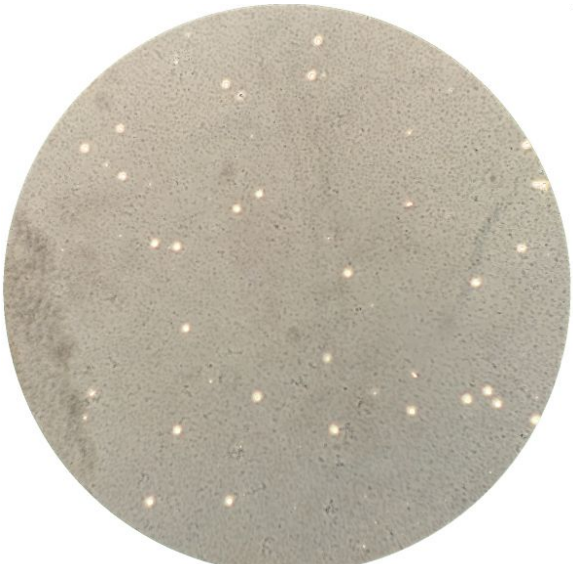

Fig. 3b | Example: unacceptable cell density (*200x*)

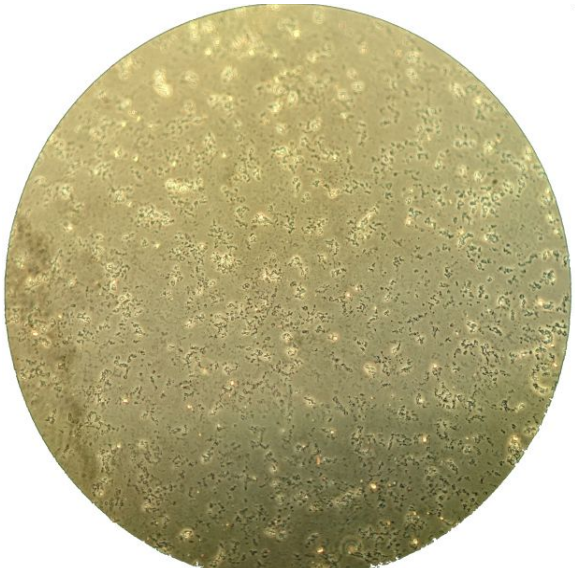

Fig. 3c | Example: Dirt, no cells (*200x*)

E. Troubleshooting

Problem	Possible cause	Solution
No defined PBMC layer (Protocol step **A.4.**)	Incorrect adapter size	Use 12x75 mm centrifuge tube adapter.
	Centrifuge not calibrated	Calibrate correctly.
	Centrifugation speed too low	Increase speed to 465 g.
	Centrifugation time too short	Increase centrifugation time (up to 30 min).
Reddish PBMC layer (Protocol step **A.4**)	Blood sample too old	Process blood within 8 hours after collection.
	Blood volume too high	Decrease sample volume to 1 ml.
Air bubbles in the chip (Protocol step **B.3.**)	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 (Protocol step **B.4.**)	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 (Protocol step **B.6.**)	Low cell count	If possible, reload the chip with 100 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.