



- Exploratory Protocol ZKWSOP 0010-

Preparation of Leukocytes from CSF for Chipcytometry

Short Description

This SOP describes how to isolate leukocytes from CSF for biobanking and analysis by Chipcytometry.




Disclaimer

This protocol is has not been validated for clinical use and its exploratory in nature. All protocol development has been done on samples from healthy subjects. A disease specific validation is strongly recommended.

Versioning

| | | |
|------------------|-----|-------------------|
| Version Number | 1.3 | valid from: 2018- |
| Replaces Version | 1.2 | from: 2018-04-11 |

Signatures

| | | |
|------------------------------|--------------------|---------------------------------------------------------------------------------------------------|
| Author Nancy Stanslowsky | Date 2018-11-20 | Signature  |
| Reviewer Anja Mirenska | Date 2018- | Signature  |
| Approval Christian Hennig | Date 2018- | Signature  |

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 CSF, and how the cells are loaded and fixed on ZellSafe™ chips for subsequent testing.

B. Definitions and Abbreviations

- CSF: Cerebrospinal fluid

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 pelleted from CSF by 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

- Minimum of **9 ml CSF**, not cooled, not stored longer than 30 min.

Equipment and Materials

- Pipettes and pipette tips (non-sterile)
- Centrifuge with swinging bucket rotor
- 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 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| ZellSafe™ Cell Chips (Cat.# 28050606/01-010) 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

Process CSF samples as quickly as possible (time till chip loading preferably < 30 min).

A. Sample preparation

NOTE: To avoid cell loss, use tubes with non-adhesive surfaces and V-shaped bottom.

1. Centrifuge a minimum of 9ml CSF in a swinging-bucket rotor (**10 min; 1000 g; RT; Acc. 9; Dec. 9**).
2. Decant the supernatant into a separate 15 mL Sarstedt tube and aliquot into approximately 0.4 mL aliquots into 0.5 mL screw-top tubes. Resuspend the cell pellet in 10-30 μ L of the remaining CSF volume. Please be aware, that the pellet might not be visible.

B. Preparation and loading of the ZellSafe™ chips

1. Apply the patient identification label (optional; not included in the kit) on the ZellSafe™ chip at the position indicated in Fig. 2a . 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. 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 μ l **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 the entire cell solution into the chip and allow the cells to settle (**10 min; RT**).
6. Rinse the chip with 5x 200 μ l **ZELLKRAFTWERK wash buffer** and **verify cell density** within the first third of the chip with a standard light microscope. Please refer to section “C. Quality control” for examples.
7. Rinse the chip with 5x 200 μ l **ZELLKRAFTWERK fixation buffer**. Incubate **45 min at 4°C/ 39°F**.
8. Rinse the chip with 5x 200 μ l **ZELLKRAFTWERK wash buffer**.
9. For storage rinse the chip with 5x 200 μ l **ZELLKRAFTWERK storage buffer**.
10. Tightly seal the chip with the sealing plugs. First seal the outlet, thereafter the inlet.



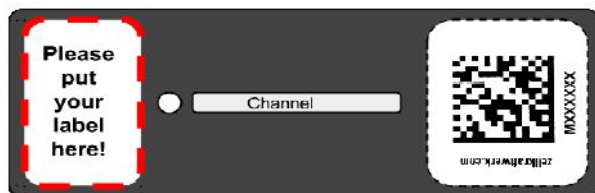


Fig. 2a | Space for additional label on the ZellSafe™ chip (label not included).



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

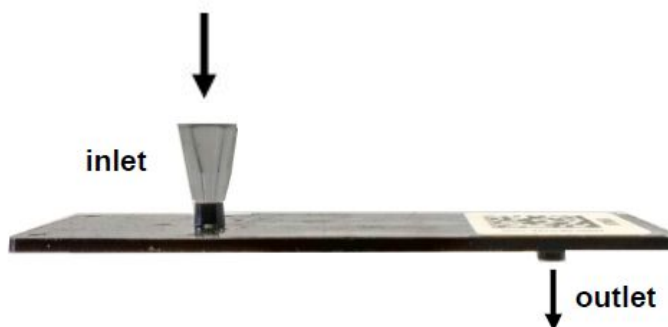


Fig. 2c | Zell Safe™ chip with pipette adapter.

Note: The ZellSafe™ chips that are to be shipped should be stored in a ZellSafe™ box at **4°C/ 39.2°F**. The shipping conditions are 4°C/ 39.2°F with temperature tracking. **DO NOT FREEZE!** Please complete the attached sample manifest and place it to the shipment. Also, please ship the samples **within 6 weeks** after preparation 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 9).

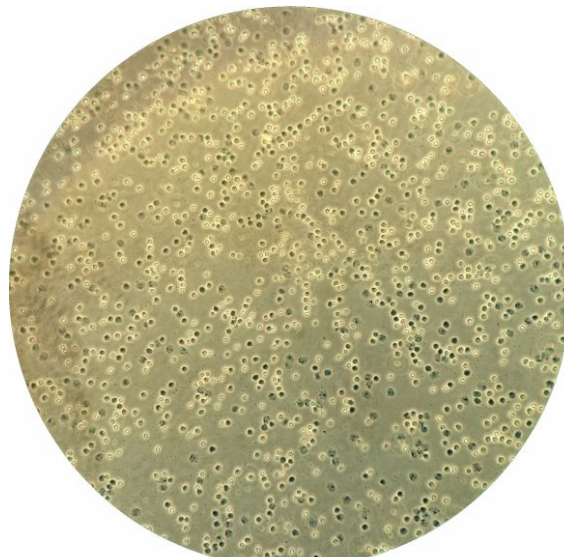


Fig. 3a | Example: acceptable cell density (200x)

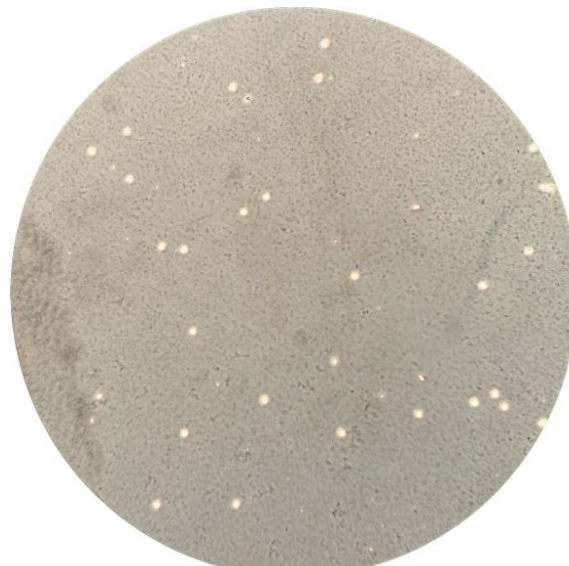




Fig. 3b | Example: unacceptable cell density (200x)

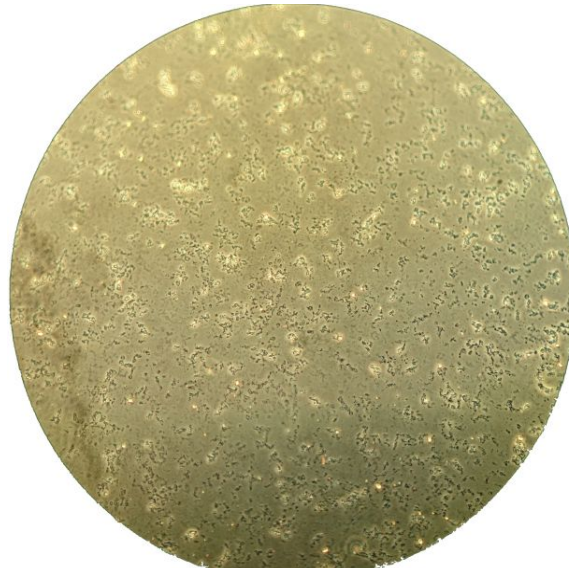


Fig. 3c | Example: Dirt, no cells (200x)





E. Troubleshooting

| Problem | Possible cause | Solution |
|------------------------------------------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------|
| 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 50 μ l 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 | |
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| 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.