- Standard Operating Procedure -

Preparation and fixation of human BAL cells on ZellSafe_C chips

Short Description

This SOP describes how to prepare and fix cells from broncho-alveolar lavage from patients on ZellSafe_C chips

Versioning

Version Number	2.1	valid from: 2018-11-19
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Signatures

Author Karen Böttcher	Date 2018-11-19	Signature 
Reviewer Jan Keckeis	Date 2018	Signature
Approval Christian Hennig	Date 2018	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 cells of a human broncho-alveolar lavage are prepared for and immobilized on ZellSafe_C chips.

B. Definitions and Abbreviations

BALF: Broncho-alveolar lavage 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

Cells in BALF are pelleted by centrifugation, resuspended in buffer and pipetted into the ZellSafe_C chip, 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

- BALF

Equipment and Materials

- pipettes and pipette tips (not sterile)
- cooled centrifuge
- ZELLKRAFTWERK washing box

Reagents and solutions

- ZELLKRAFTWERK wash buffer
- ZELLKRAFTWERK lysis buffer
- ZELLKRAFTWERK storage buffer
- ZELLKRAFTWERK fixation buffer

Procedure

All steps are performed as quickly as possible at room temperature.

1. Measure cell number in BALF sample (total cells/sample)

2. Filter BAL using a 70µm filter

3. Centrifuge BALF at 320g (acc 9 dec 9) at room temperature for 10 minutes for samples with low volume up to 10ml, at 400g for 15 min for volumes >10ml

4. Optional: harvest supernatant for other experiments

5. Resuspend cell pellet in 10 ml PBS (to remove the mucus)

6. Centrifuge at 320g (acc 9 dec 9) at room temperature for 10 minutes

7. repeat the last two steps one time

8. Resuspend cell pellet:

total cell number	**resuspension volume**
<= $1x10^6$ initially counted cells	40µl
> $1x10^6$ initially counted cells	40µl per $1x10^6$ cells

Note that, although you counted 1 million cells, only a part will pellet after centrifugation. The fraction of pelleted cells depends on the inital cell density - higher cell density means higher recovery rate. So the cell number on chip will in fact be lower then 1 million cells! To recover more cells from solution by centrifugation, g-force can be set to up to 1500g.

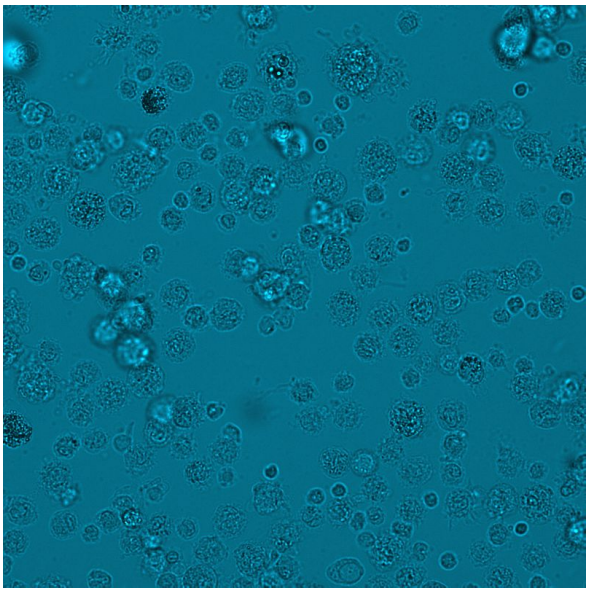
9. Remove the sealing plugs from the chips. Make sure that no air is within the cavities of the chip in- and outlets before plugging the pipetting adapter into the inlet of the chip. In this case, pipettes some drops ZELLKRAFTWERK wash buffer into the in- or outlet to remove air. Plug-in pipetting adapter to the inlet. **NEVER LET THE CHIPS RUN DRY!**

10. Apply up to 100µl of the resuspended cell solution to the ZellSafe_C chip following video instructions at http://youtu.be/aEJoXk3c1tU .

11. Incubate cells on chip for 5 minutes at room temperature

12. **CAREFULLY** rinse chip with 3x 100µl ZELLKRAFTWERK wash buffer to remove unbound cells and debris

13. Control/document cell density by microscopy



Typical cell densitiy of BAL cells on ZellSafe_C chips

13. **CAREFULLY** rinse chip with 5x 100µl ZELLKRAFTWERK fixation buffer.

14. Incubate for 15 minutes at 4°C/ 39.2°F

15. Rinse chip with 5x 100µl ZELLKRAFTWERK storage buffer for long term storage or ZELLKRAFTWERK wash buffer for short term storage

16. **TIGHTLY** seal the chip with sealing plugs.