- Standard Operating Procedure -

Preparation and fixation of short term cultured cells on ZellSafe_C chips

Short Description

This SOP describes how to prepare and fix primary cells that have been cultured for up tp 24h on ZellSafe_C chips

Versioning

Version Number	1.2	valid from: 2018-11-19
Replaces Version	1.1	from: 2013-04-17

Signatures

Author Karen Böttcher	Date 2018-11-19	Signature 
Reviewer Anke Hofmeister-Brix	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 primary cells from short term cell cultures are harvested, prepared and immobilized on ZellSafe_C chips.

B. Definitions and Abbreviations

PFA: phosphate buffered formaldehyde
PBS: phosphate buffered saline
BSA: bovine serum albumine
EDTA: Ethylenediaminetetraacetic acid

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 are pelleted by centrifugation, resuspended in relaxation-buffer and left untouched for 15 minutes. Afterwards, they are again pelleted, resuspended and pipetted into the ZellSafe_C chip following a standard procedure. Application of cellsolutions to 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

- Primary cells after short term cell culture

Equipment and Materials

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

Reagents and solutions

- ZELLKRAFTWERK relaxion buffer (PBS, 0.5% BSA, 2mM EDTA)
- ZELLKRAFTWERK storage buffer
- ZELLKRAFTWERK wash buffer
- ZELLKRAFTWERK fixation buffer

Procedure

1. Optional: Measure cell number in cell culture well (viable cells/sample)

2. Harvest cell culture fluid and centrifuge cells at 1000g (acc 9 dec 9) / 4°C (39,2°F) for 10 minutes

3. Optional: harvest supernatant for other experiments

4. Resuspend cell pellet in 500µl ZELLKRAFTWERK relaxation buffer in an Eppendorf tube

5. Leave the cells untouched for 15 minutes at room temperature

6. Gently resuspend sedimented cells and centrifuge cells at 1000g (acc 9 dec 9)/ 4°C (39,2°F) for 10 minutes

7. Discard supernatant and resuspend cell pellet in ZELLKRAFTWERK wash buffer (If you measured the cell number (step 1), here are recommendations for suitable resuspension volumes):

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

8. 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 the pipetting adapter to the inlet. **NEVER LET THE CHIPS RUN DRY!**

9. Put the ZellSafe chip in the orange frame and place the outlet of the chip over a collection reservoir to collect flowthrough/waste

10. Rinse the chip with 3x200µl ZELLKRAFTWERK wash buffer prior to use (remove any air bubbles)

11. Apply the respective volume of the cell solution from step 7 to the ZellSafe chip following video instructions at http://youtu.be/aEJoXk3c1tU .

12. Incubate cells on chip for 5 minutes (both room temperature and 4°C (39,2°F) are possible)

13. **CAREFULLY** rinse chip with 2x 200µl ZELLKRAFTWERK wash buffer to remove unbound cells and debris. The most gentle way of doing this is to apply the fluid dropwise on top of the pipetting adapter

14. Optional: control/document cell density by microscopy

15. **CAREFULLY** rinse chip with 2x 200µl ZELLKRAFTWERK fixation buffer

16. Incubate for 45 minutes at 4°C/ 39.2°F

17. Rinse chip with 4x 200µl ZELLKRAFTWERK storage buffer for long term storage or ZELLKRAFTWERK

wash buffer for short term storage

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