- Standard Operating Procedure -

Preparation of leukocytes from lung cell culture cells

Short Description

This SOP describes how to prepare leukocytes after lung cell culture from dissociated lung tissues for chipcytometry

Versioning

Version Number	1.1	valid from: 2018-11-19
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Signatures

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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 from lung tissue are prepared for culture and how PBMC from these cultures are prepared for fixation on ZellSafe_C chips..

B. Definitions and Abbreviations

- PBMC: peripheral blood mononuclear cells
- FACS: fluorescence-activated cell sorting
- PBS: Phosphate-buffered saline
- PFA: Paraformaldehyde
- MACS: magnet-activated cell sorting

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

Lung cells are mechanically separated from lung tissue and cultured. PBMC are separated from the content of the cell culture wells by Ficoll gradient centrifugation, resuspended in buffer and pipetted into the ZellSafe_C chip following a standard procedure. Application of cellsolutions to ZellSafe_C chips is demonstrated here: 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

- Lung cell culture

Equipment and Materials

- pipettes and pipette tips (not sterile)
- 70µm cell strainer
- insuline syringe (use syringe stamp)
- cellculture wells
- centrifuge
- FACS-tubes (BD Falcon, Cat# 352058)
- 15 ml tubes

Reagents and solutions

- Bicoll (Ficoll) (Biochrom, Cat# L6115)
- ZELLKRAFTWERK wash buffer
- ZELLKRAFTWERK storage buffer
- ZELLKRAFTWERK fixation buffer
- MACS buffer (PBS + 0.5% BSA + 2mM EDTA)

Procedure

1. place lung tissue in a cell strainer (70 µm) inside a 6-well plate

2. dissociate lung tissue mechanically

3. press the dissociated lung tissue through the cell strainer using a syringe stamp
4. Do lung cell culture following the respective SOPs
5. after lung cell culture, transfer cell solution from cell culture well(s) to a 15ml tube
6. wash well with 3-4 ml PBS and add to tube from step 5
7. centrifuge for 10 min at 1000g at room temperature
8. remove 8ml of supernatant (leaving 2 ml inside the tube)
9. add 1 ml of MACS-Puffer and resuspend cell pellet
10. pipette 2.25ml Ficoll to a FACS-tube,
11. **CAREFULLY** overlay 3ml of the solution from step 9 over the Ficoll phase. Take care that the two phases are not mixing
12. centrifuge for 10 min at 465g (accelaration 7, deceleration 1) at room temperature
13. harvest the opaque cell-containing interphase into a new FACS-tube and resuspend with 1ml PBS
14. centrifuge this suspension for **10 minutes, 100g (**acc 9 dec 9)**, room temperature**
15. discard the supernatant completely (contains debris)
16. 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!**
17. resuspend the cell pellet in **appropriate volume ZELLKRAFTWERK wash buffer (min volume 10µl)**, pipette to the chip and allow the cells to settle for 5 minutes
18. **CAREFULLY** rinse chip with 3x 100µl ZELLKRAFTWERK storage buffer
19. **CAREFULLY** rinse chip with 5x 100µl ZELLKRAFTWERK fixation buffer

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

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

22. **TIGHTLY** seal the chip with mini luer plugs