- Standard Operating Procedure 0005-

Preparation and fixation of tissue cryosections on ZellSafe_T chips

Short Description

This SOP describes how to prepare and fix tissue cryosections on ZellSafe_T chips

Versioning

Version Number	1.5 New desired section thickness: 7µm	valid from: 2018-06-14
Replaces Version	1.4	from: 2017-03-27 and older

Signatures

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Approval Christian Hennig	Date 2018-06-14	Signature

Target Group

	lab personnel Zellkraftwerk; customers Zellkraftwerk
internal use only	No, available to third parties

Table of Content

A. Scope

The scope of this standard operating procedure is to describe how tissue is prepared for Chipcytometry by cryosectioning and how tissue cryosections are fixed on ZellSafe_T chips.

B. Definitions and Abbreviations

C. Personnel Qualifications

Basic education and training

- handling of microtoms
- 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

In regard to biomarker stability, cryosections are superior to Paraffin-embedded tissue. Therefore, we recommend only to use cryosections in Chipcytometry. Sections are cut by using a Cryomicrotom on 24x50mm coverslips. The coverslip is then put on ZellSafe_T chips using the double-sided adhesive tape. The complete procedure for cryosections is demonstrated in this video: http://www.youtube.com/watch?v=SNJAxOU6F5k . **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

- Tissue

Equipment and Materials

- cryomicrotome
- optional: rotator or rocker/shaker to incubate tissue on coverslip to remove Tissue-Tek
- coverslips 24x50
- Coplin Staining Jar (e.g. BRAND glass staining trough, Coplin pattern, Sigma-Aldrich, # BR472800)
- ZellSafe Tissue chips

Reagents and solutions

- ZELLKRAFTWERK wash buffer
- ZELLKRAFTWERK storage buffer
- ZELLKRAFTWERK fixation buffer
- Ethanol 70%

Procedure

(Special preparation for lung tissue: Cryoprotection
- Flush the tissue with ZELLKRAFTWERK fixation buffer and incubate on a shaker for 5h at 4°C/39°F in ZELLKRAFTWERK fixation buffer
- Change buffer to 30% sucrose in PBS and incubate for 24h at 4°C/39°F)

1. Snap-freeze tissue at -20°C/-4°F (if necessary, embed tissue in OCT/Tissue-Tek prior to freezing)

DO NOT FIX TISSUE BLOCKS AT THIS STAGE IN PFA OR OTHER FIXATIVES.

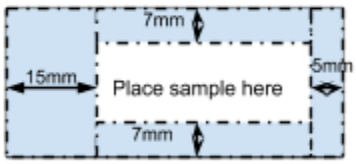
(Optional: Freeze at -80°C/-112°F for long term storage prior to sectioning)

2. Prepare cryosections using a cryomicrotome (7µm section thickness)

3. Transfer the section directly onto a 24x50mm coverslip. **Note: Never** pretreat the coverslip with any solvents, e.g. ethanol!

4. **DON'T LET THE SECTION DRY ON THE COVERSLIP** (this will degrade biomarkers) .

Immediately transfer the coverslip to cooled (4°C/39°F) Zellkraftwerk fixation buffer vertically in the Coplin Staining Jar and fix the biomarkers for 45min at 4°C/39°F. Afterwards, carefully rinse the coverslip for 5 minutes into Zellkraftwerk wash buffer to remove fixation buffer and remaining OCT. Dry the borders (NOT THE SECTION!) of the coverslip with a lint wipe to enable attachment to the chip.



5. Prior to use, clean the glass surfaces of the microfluidic chip the ZellSafe_T chips using ethanol solution and a **lint** wipe. Please don't touch the inside glass surface of the channel afterwards.

6. Remove the protection film from the double-sided adhesive tape on the ZellSafe_T chip using the colored badge.

7. Make sure that the glass of the coverslip is dry. Place the coverslip, the tissue section facing the channel, on the adhesive tape.

8. Place the pipetting adapter on one of the inlets of the chip.

9. Rinse the chip with 2x1ml ZELLKRAFTWERK wash buffer. **NEVER LET THE CHIPS RUN DRY!**

10. Remove the pipetting adapter. **TIGHTLY** seal the chip with 2 mini luer plugs at the entry and the outlet of the chip.