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Tissue Cryosection, Preparation and Fixation on ZellSafe™-T Chip Using ZKW Fixation Buffer Quick Guide

Below are guidelines for how tissue is prepared for ChipCytometry by cryosectioning using a cryomicrotome and fixating using ZKW Fixation Buffer, followed by adhesion to ZellSafe™ Tissue chips.

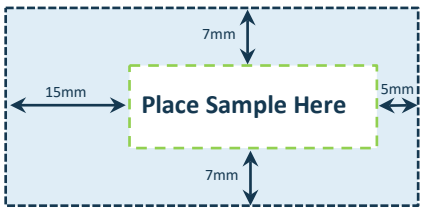

Fig. 1 | Coverslip Area for Sample Placement (Size is to scale)

Note: For some tissues, it might be advantageous to mount the tissue sections on coverslips with tissue adhesive surface coating. Coverslips with adhesive coating are available at ZELLKRAFTWERK.

A. Tissue Cryosection Preparation

1. Snap-freeze tissue at -20°C/-4°F. If necessary, embed the tissue in OCT/Tissue-Tek prior to freezing. Refer to Canopy Quick Guide – OCT Embedding of Tissue Samples for Cryosectioning
   - Optionally, you can freeze the tissue at -80°C/-112°F for long term storage prior to sectioning
2. Prepare the cryosections using a cryomicrotome to create sections 5-7µm in thickness.
3. Transfer the section directly onto a 24x50mm coverslip.

Never pretreat the coverslip with any solvents including ethanol

<u>Special Prep for Lung Tissue Cryoprotection:</u>

1. Flush the tissue with ZKW Fixation Buffer and incubate on a shaker for 5 hours at 4°C/39°F in ZKW fixation buffer
2. Change buffer to 30% sucrose in PBS and incubate for 24 hours at 4°C/39°F

B. Tissue Fixation

All solutions for fixation **must be at 0-4ºC or on ice** for the entire fixation process.

Do not let the section dry on the coverslip!

1. Prior to cryosectioning, fill a Coplin Staining Jar with ZKW Fixation buffer and cool to 4°C/39°F.
2. Immediately transfer the sectioned tissue on coverslip into the Coplin Jar vertically and fix the biomarkers for 45 minutes at 4°C/39°F.
3. Remove the coverslip from the jar and rinse the coverslip for 5 minutes in ZKW wash buffer.
4. Carefully dry the borders, **not the section**, of the coverslip with a lint-free wipe to enable attachment to the chip. Do not let tissue dry out!

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ChipCytometry Tissue Quick Guide

C. ZellSafe™-T Chip Preparation

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 2 (optional; not included in the kit). Please do not write on the QR-code label.

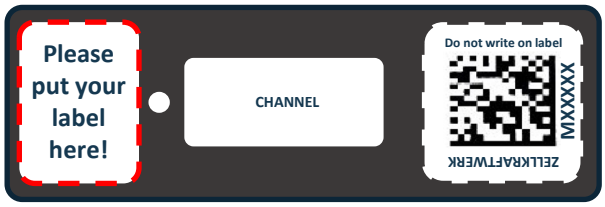

Fig. 2 | Space for additional label on ZellSafe™ chip
(Label not included)

2. Carefully clean the inner glass surface of the ZellSafe™-T Chip using an ethanol solution and lint-free wipe. After cleaning, take care not to touch the glass surface of the channel.
3. Remove the protective film from the adhesive surface on the bottom of the ZellSafe™-T Chip. Tweezers/forceps may be useful in film removal.
4. Align the coverslip onto the adhesive surface of the chip with the tissue section facing the channel opening.
5. Apply gentle pressure to the coverslip above the adhesive surface to minimize air lock between the chip and the coverslip.

DO NOT apply pressure on the coverslip glass directly above the channel!

6. Place the pipetting adapter on the inlet of the chip (Fig. 3) and rinse the chip with **2x 1 ml ZELLKRAFTWERK sterile storage buffer**. Sterile storage buffer should always be used to avoid contamination.

Never let the chip run dry! Avoid pipetting air bubbles through the chip channel!

7. TIGHTLY seal the chip with 2 mini luer plugs at the outlet and inlet of the chip. Store at 4ºC/39ºF.

Note: ZellSafe™-T chips that are to be shipped should be stored in a ZellSafe™ box. The shipping conditions are 4°C/ 39.2°F with temperature tracking (RFID). **DO NOT FREEZE!**

Fig. 3 | ZellSafe™ chip with pipette adapter

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