

Tissue Cryosection Preparation for ZellSafe™ Tissue Chip Using AC/EtOH Quick Guide

Below are guidelines for how tissue is prepared for ChipCytometry by treating cryosections with acetone and ethanol for fixation, followed by adhesion to ZellSafe™ Tissue chips.

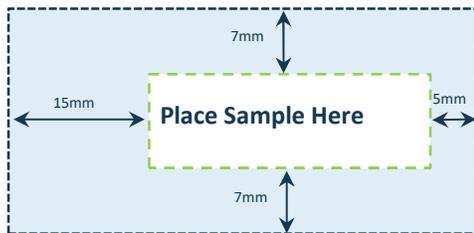


Fig. 1 | Coverslip Area for Sample Placement
(Size is to scale)

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. Mounting Tissue Cryosection to Coverslip

1. Prepare tissue cryosections with a thickness of **5-7 μm** at a cryomicrotome.

Note: For biomarker stability, cryosections are strongly recommended (when possible) over paraffin-embedded tissue.

2. Place the sectioned tissue onto a **24x50mm** (thickness No. 1) coverslip in the area indicated by Fig. 1.

NEVER let the section thaw!

3. After tissue placement, coverslips should be stored at **-80°C** for 12-24 hours before fixation.

B. Tissue Fixation

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

Do not let the section dry on the coverslip!

1. Prepare four Coplin staining jars (Fig. 2) and separately fill each with acetone, 90% ethanol, 70% ethanol, and ZELLKRAFTWERK wash buffer.
2. Chill the Coplin staining jars at 0-4°C or on ice prior to tissue fixation steps.
3. Remove the coverslip from -80°C freezer, immediately put it into the acetone filled Coplin staining jar and incubate for exactly **5 minutes**.
4. Remove the coverslip from the Acetone filled jar, immediately put it into a 90% ethanol filled jar, and incubate for exactly **3 minutes**.

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B. Tissue Fixation continued...

5. Remove the coverslip from the 90% ethanol filled jar, immediately put it into the 70% ethanol filled jar, and incubate for exactly **3 minutes**.
6. Remove the coverslip from the 70% ethanol filled jar, immediately put it into the ZELLKRAFTWERK wash buffer filled jar and incubate for exactly **6 minutes**.

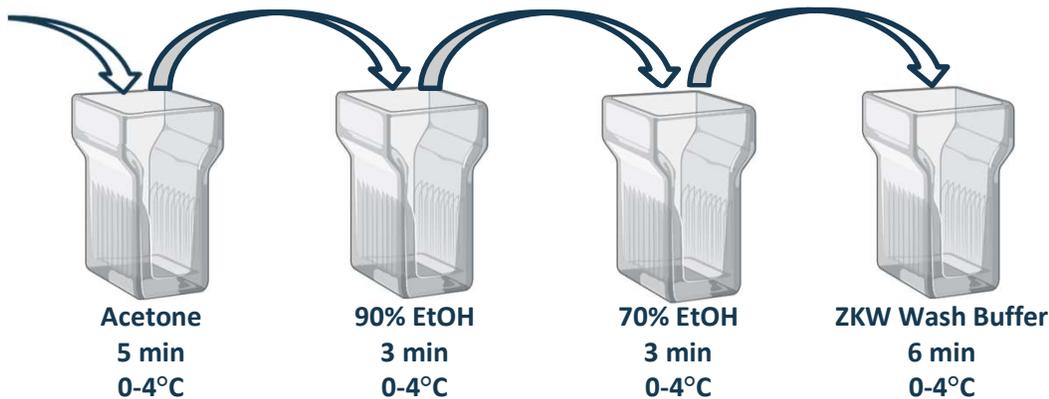


Fig. 2 | Illustration of Tissue Fixation on Coverslip through Coplin Staining Jars
(Image created using BioRender)

C. ZellSafe™-T Chip Preparation

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 3 (optional; not included in the kit). Please do not write on the QR-code label.
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. On the coverslip, dry the borders around the tissue section with a lint-free wipe to enable attachment to the chip. Take care not to touch the tissue section, and do not let the tissue dry out!

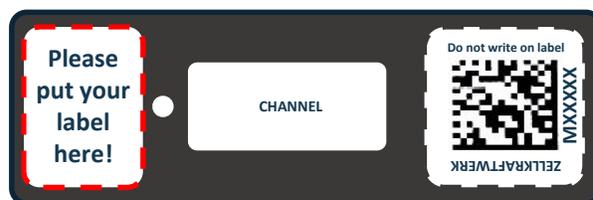


Fig. 3 | Space for additional label on ZellSafe™ chip
(Label not included)

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C. ZellSafe™-T Chip Preparation continued...

5. Align the coverslip onto the adhesive surface of the chip with the tissue section facing the channel opening.
6. 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!

7. Place the pipetting adapter on the inlet of the chip (Fig. 4) 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!

Note: Exchange with fresh, sterile ZKW storage buffer after approximately one year to prevent contamination.

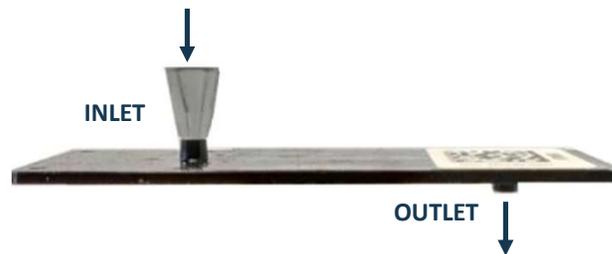


Fig. 4 | ZellSafe™ chip with pipette adapter

8. TIGHTLY seal the chip with 2 mini luer plugs at the outlet and inlet of the chip.
9. Store the chip at 4°C.

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!**