Sample Preparation of Fresh Frozen Tissue

Below are guidelines for how tissue samples are prepared for CellScape by treating cryosections with acetone and ethanol for fixation, followed by adhesion to slides for use with CellScape Whole-Slide Imaging Chambers (WSIC).

Materials & Reagents

- ☐ Cryomicrotome (Cryostat)
- ☐ Tweezers
- ☐ Tissue-Tek® OCT Compound
- ☐ Acetone
- ☐ Ethanol (90% & 70%)
- ☐ Coplin Staining Jar (e.g., Sigma-Aldrich Cat. # BR472800)
- ☐ Pipette
- ☐ Pipette tips
- ☐ Glass Slides (1 mm thick, 74-76 mm x 24-26 mm; e.g., Fisher Scientific Cat.# 22-035813)

Bruker Item	Size	Catalog #	Contents
CellScape Wash Buffer	500 mL	PRSM-BUF-WASH-500mL	500 mL Wash Buffer

Before You Start

- Follow good laboratory practices and maintain a clean environment when working with samples.

Step 1: Mounting Tissue Cryosection to Slide

a. Prepare tissue cryosections with a thickness of **7 µm** using a cryomicrotome.

b. Place the section onto a standard microscope slide in the area indicated by Figure 1.
Note: Any tissue outside this area will not be scanned and may disrupt adhesive border.
Note: Never let the section thaw.

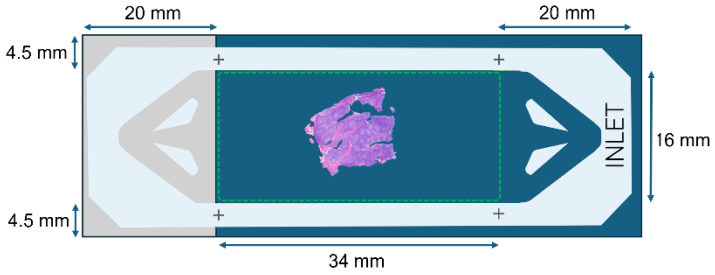

Figure 1. Placement of cryo-sectioned tissue onto an example frosted glass slide for compatibility with overlaid CellScape WSIC. The CellScape viewing window is indicated by the green dashed line. Tissue is ideally centered on the glass slide and does not encroach into the adhesive area on the border.

c. After tissue placement and prior to fixation, slide should be stored at -80 °C for 12-24 hours to improve tissue adherence to coverslip

Step 2: Tissue Fixation

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

Note: Do not let the section dry on the slide as tissue adherence may be compromised.

a. Prepare four Coplin staining jars (Figure 2) and separately fill each with acetone, 90% ethanol, 70% ethanol, and CellScape Wash Buffer. (*Optional*: pre-chill containers to 4 °C for 10 min).

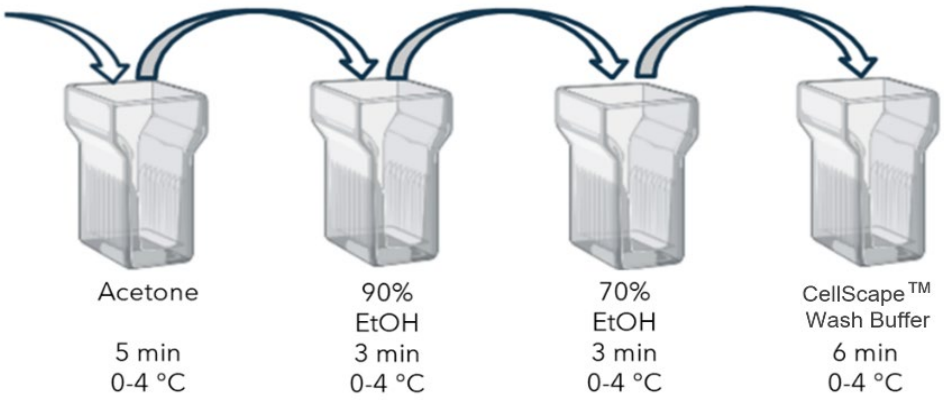

Figure 1. Illustration of tissue fixation on coverslip using Coplin staining jars.

b. Before proceeding to steps c-f, ensure the filled Coplin staining jars are maintained at **0-4 °C or on ice**.

c. Remove the slide from -80 °C and immediately put it into the **acetone** filled jar for **5 minutes.**

d. Remove the slide from acetone and immediately put it into the **90% ethanol** jar for **3 minutes**.

e. Remove the slide from 90% ethanol and immediately put it into the **70% ethanol** jar for **3 minutes**.

f. Remove the slide from 70% ethanol and immediately put it into the **CellScape Wash Buffer** jar for **6 minutes**.

g. Remove the slide from CellScape Wash Buffer.

h. To facilitate slide adhesion to the WSIC coverslip, use a lint-free wipe to dry the borders of the slide where the adhesive of the WSIC coverslip will contact the slide (see Figure 1). Take care not to touch the tissue section and do not let the tissue dry out as tissue adherence may become compromised.

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