Sample Preparation of Formalin-Fixed Paraffin Embedded Tissues

Below are guidelines outlining FFPE tissue section preparation for CellScape assays, including deparaffinization and antigen retrieval in preparation for assembly of CellScape Whole-Slide Imaging Chambers (WSIC).

Materials & Reagents

- ☐ Standard rotary or sledge microtome for FFPE sectioning
- ☐ Temperature controlled water bath (≥95 °C) for antigen retrieval
- ☐ Plastic racks (Carl Roth #HA49.1) and jars (Carl Roth #HA48.1) for antigen retrieval
- ☐ Jars for deparaffinization (e.g., Carl Roth #ETN7.1, 250 ml)
- ☐ Ethanol 100%, 90%, 70%, 50% (e.g., Sigma-Aldrich Cat. # 1009742511)
- ☐ Histo-Clear II (EMS, Cat #. 64111-01) or Roticlear (Carl Roth A538.5) or Xylene/Xylene alternative
- ☐ Antigen retrieval buffer Cell Conditioning 1 (CC1) (Roche, Cat. # 950-124)
- ☐ Glass Slides (1 mm thick, 74-76 mm x 24-26 mm; e.g., Fisher Scientific Cat.# 22-035813)
- ☐ Pipette and tips
- ☐ Fume hood

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: Preparing FFPE Tissue Sections

a. Using a rotary or sledge microtome, cut **5 µm** thick sections from Formalin-Fixed Paraffin-Embedded (FFPE) tissue blocks.

b. Float sections in a 37 °C water bath and capture on glass slides in the area indicated in Figure 1 to ensure compatibility with the CellScape WSIC viewing window. Any tissue located outside the indicated area will not be scanned and may disrupt adherence of the chamber glass.

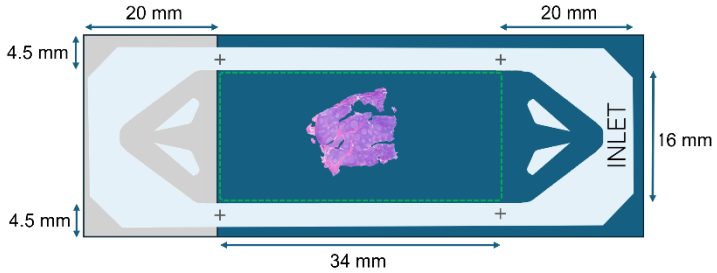

Figure 1. Placement of FFPE tissue onto an example frosted glass slide for compatibility with overlaid CellScape WSIC. The CellScape viewing window is indicated by the green dashed line. Tissue is ideally centered on the glass slide and does not encroach into the adhesive area on the border.

c. Allow the tissue-mounted slides to drain in an inclined position for about 15 minutes to eliminate remaining liquid underneath the tissue section.

d. To remove residual moisture, bake the sections at 60 °C overnight.

e. Coverslips with tissue sections can now be stored at room temperature or 4 °C. Samples are now ready for subsequent deparaffinization, rehydration, and antigen retrieval.

Step 2: Deparaffinization

All solutions for deparaffinization should be maintained at **room temperature** for the entire deparaffinization process.

a. Working under a fume hood, prepare and label 10 staining jars filled with 100 mL of the following solutions:
   1. Histo-Clear II Jar 1
   2. Histo-Clear II Jar 2
   3. Histo-Clear II Jar 3
   4. 100% ethanol (EtOH) Jar 1
   5. 100% ethanol Jar 2
   6. 90% ethanol
   7. 70% ethanol
   8. 50% ethanol
   9. 30% ethanol
   10. CellScape Wash Buffer

b. Incubate the tissue-mounted slides in each jar, **in numerical order**, for 5 minutes per solution.

c. Leave the rack of tissue-mounted slides in the CellScape Wash Buffer container until ready to be transferred to the antigen retrieval solution container in the next step.

Note: Histo-Clear II and ethanol used in Jar 1 can be reused when kept in sealable containers.

Note: Prepare fresh Histo-Clear II after approximately 15-20 sections have been deparaffinized.

Note: Prepare fresh ethanol after approximately five sample processing rounds, independent of the number of sections that have been processed.

Note: Never let the sections run dry after deparaffinization as tissue adherence may be compromised.

Step 3: Antigen Retrieval

a. Heat a separate jar of CC1 antigen retrieval solution in a water bath or pressure cooker to 95 °C.

b. Place the rack with tissue-mounted slides in the solution for 20 minutes.

c. Transfer the sections to room-temperature CellScape Wash Buffer for at least 5 minutes.

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