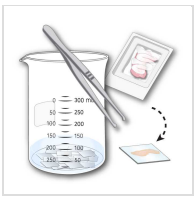

May 24, 2022

CODEX® Multiplexed Imaging | Tissue Sectioning

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Vanderbilt Diabetes Research Center

Islet and Pancreas Analysis Core
Vanderbilt Diabetes Research Center

This protocol is adapted from the CODEX User Manual, revision C (Akoya Biosciences, Dec. 2020). See also: Tissue Processing - Best Practices.

This protocol describes the tissue preparation processes for the CODEX® (now PhenoCycler™) system by Akoya Biosciences. For the comprehensive multiplexed imaging workflow currently in use at the Vanderbilt Diabetes Research Center, please see **CODEX® Multiplexed Imaging | Modality overview**.

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protocol ,

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Apr 24, 2022 Diane Saunders Vanderbilt University

May 10, 2022 Islet and Pancreas Analysis Core Vanderbilt Diabetes Research Center

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In steps of
CODEX® Multiplexed Imaging | Modality Overview

Poly-l-lysine, 0.1% (wt/vol) **Sigma Aldrich Catalog #P8920** Step 1

22 x 22m Glass Cover Slips # 1 1/2 **Electron Microscopy Sciences Catalog #72204-01** Step 1

Storage:

- PolarSafe™ PCR Cardboard Freezer Boxes **Fisher Scientific Catalog #10-987-065** Step 13
  7x8 Double Zipper
- Bags **Uline Catalog #S-20325** Step 13
  6x9 Double Zipper
- Bags **Uline Catalog #S-17788** Step 13

Equipment:

CM1950 cryostat
Leica **CM1950**

:

This protocol is adapted from the CODEX User Manual, revision C (Akoya Biosciences, Dec. 2020). See also: Tissue Processing - Best Practices.

Coverslip Preparation

1 Gather reagents:

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- Poly-l-lysine, 0.1% (wt/vol) **Sigma Aldrich Catalog #P8920**
- 22 x 22m Glass Cover Slips # 1 1/2 **Electron Microscopy Sciences Catalog #72204-01**

2 Gently place coverslips at the bottom of a 500-mL glass beaker and swirl to spread the coverslips out. Add approximately **20 mL** poly-L-lysine solution to the beaker, ensuring coverslips are fully submerged. Rotate the beaker at a 45° angle for **00:01:00** to mix.

3 Cover beaker with parafilm and incubate for minimum of **12:00:00** at **Room temperature** . Coverslips can sit in the poly-L-lysine solution for up to one week.

4 Slowly pipet off the poly-L-lysine solution and dispose.

5 Fill the beaker containing the coverslips to half volume with Milli-Q® water. Swirl gently, let sit for **00:00:30** , and then slowly pour off water into the sink.

6 **go to step #5** and repeat for a total of 5-7 washes.

7 Spread WypAll® towels on the benchtop. In small batches, remove coverslips from the beaker, separate from one another, and place individual coverslips side by side on towels. Layer another towel on top and gently dab to remove excess liquid. Leave on benchtop until completely dry.

ⓘ Coated coverslips can be stored in a petri dish for up to 2 months.

Cryosectioning

8 Set the cryostat temperature to **-20 °C** . Remove a fresh blade, clean with ethanol and a lint free wipe as needed, and insert blade.

9 Using OCT or CMC, mount sample onto the cryostat chuck in the desired orientation. Trim CMC using larger section increments of 30-50 µm until the tissue becomes visible (**Figure 1a**).

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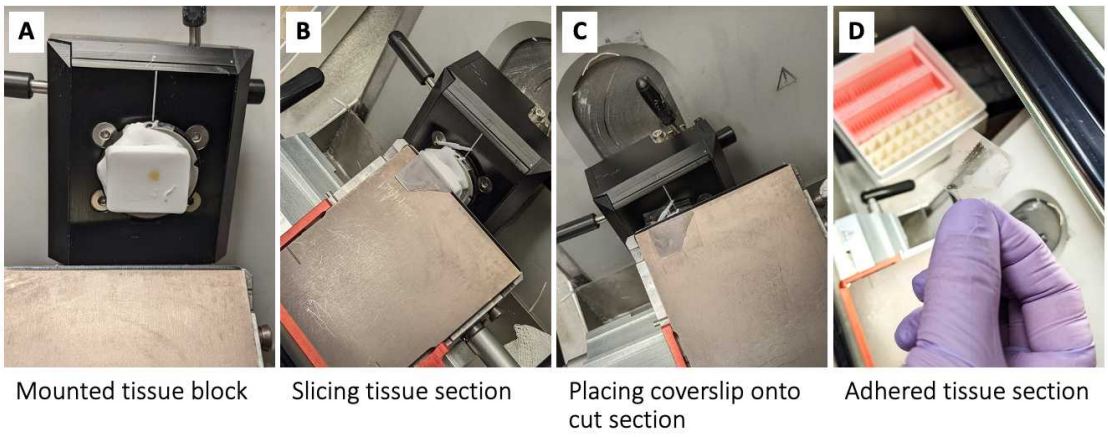

Figure 1. Sectioning cryopreserved tissue onto CODEX coverslip.

10 Obtain **6 µm** to **10 µm** sections, using a fine-tipped paintbrush to guide the section onto base and place coverslip onto section (**Figure 1b-1d**).

11 Using forceps, slot coverslips with mounted tissue into a plastic coverslip holder under the appropriate donor/block ID label(s).

12 As necessary, mount adjacent sections onto Gold Plus slides (for traditional immunohistochemistry) and/or ITO slides (for mass spectrometry). Record all sections cut, the thickness, and any additional block shaved off in the process.

Coverslip Storage

13 3D printed coverslip holders: **Codex Rack MkIII-Body.stl**

We use these with the following:

- PolarSafe™ PCR Cardboard Freezer Boxes **Fisher Scientific Catalog #10-987-065**
- 7x8 Double Zipper Bags **Uline Catalog #S-20325**
- 6x9 Double Zipper Bags **Uline Catalog #S-17788**

14 Store coverslips at **-80 °C** for up to 6 months.

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