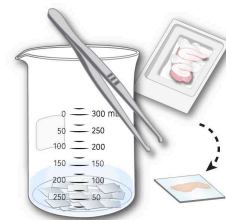


May 25, 2022

## CODEX® Multiplexed Imaging | Tissue Sectioning

DOI

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** April 25, 2022

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**Keywords:** comprehensive multiplexed imaging, tissue preparation, imaging, tissue preparation process, tissue, use at the vanderbilt diabetes research center, system by akoya bioscience, vanderbilt diabetes research center, akoya bioscience

## Disclaimer

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

## Abstract




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**.

## Materials

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

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

### Storage:

-  PolarSafe™ PCR Cardboard Freezer Boxes **Fisher Scientific Catalog #10-987-065**
-  7×8 Double Zipper Bags **Uline Catalog #S-20325**
-  6×9 Double Zipper Bags **Uline Catalog #S-17788**

### Equipment:

Equipment		
CM1950 cryostat		NAME
Leica		BRAND
CM1950		SKU



## Protocol materials

✕ 6×9 Double Zipper Bags **Uline Catalog #S-17788**

✕ Poly-l-lysine, 0.1% (wt/vol) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8920**

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## Troubleshooting

## Coverslip Preparation

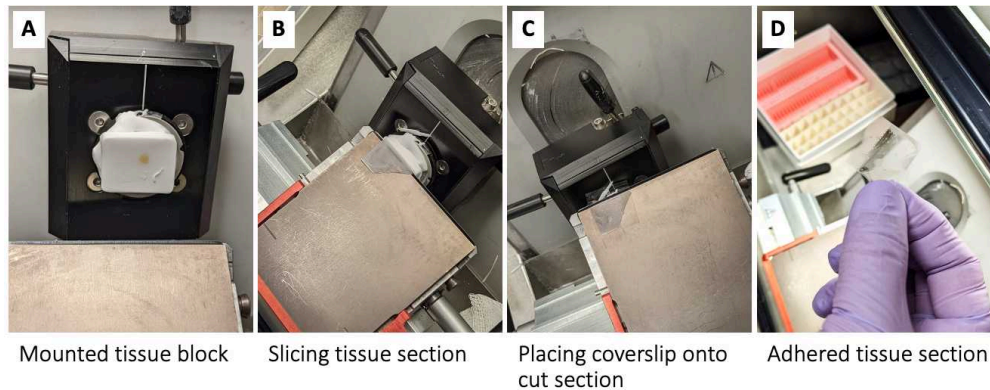
- 1 Gather reagents:
  -  Poly-L-lysine, 0.1% (wt/vol) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8920**
  -  22 × 22mm Glass Cover Slips # 1 1/2 **Electron Microscopy Sciences Catalog #72204-01**
- 2 Gently place coverslips at the bottom of a 500-mL glass beaker and swirl to spread the coverslips 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  $\mu\text{m}$  until the tissue becomes visible (**Figure 1a**).






**Figure 1. Sectioning cryopreserved tissue onto CODEX coverslip.**

- 10 Obtain  $\pm 6 \mu\text{m}$  to  $\pm 10 \mu\text{m}$  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**
-  7×8 Double Zipper Bags **Uline Catalog #S-20325**
-  6×9 Double Zipper Bags **Uline Catalog #S-17788**

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

