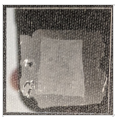

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HuBMAP - Tissue Sectioning for CODEX Specimens

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1 Works for me dx.doi.org/10.17504/protocols.io.979h9r6

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

This method details our microtomy (sectioning) process for research specimens involved with CODEX diagnostics.

This process follows the CODEX microtomy steps that follow after receiving, processing, and embedding of research specimens into formalin fixed, paraffin embedded tissue blocks.

For best results, the tissue should be devoid of folds and tears. It is critical to meet the exact measurements of 1cm x 1 cm, and be no more than a thickness of 10 µm due to the the auto-focusing capabilities of the microscope.

FFPE tissues for CODEX® analysis must sectioned onto poly-lysine coated coverslips. These tissue sections can be stored at 4° C for up to one(1) month prior to staining.

GUIDELINES

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

MATERIALS

NAME	CATALOG #	VENDOR
Water		
KimWipes		Fischer Scientific
Shandon™ Cartilage Curved Thumb Forceps, Curved, Fine Point, standard, 5 in. (12.7cm)	1631TS	Thermo Fisher
Flotation Bath	View	Fisher Scientific
Microtome Blades (Thermo Scientific Ultra)	3053835	Thermo Scientific
Gauze 4x4 Non-Sterile Squares	MSD-1400249	Fisher Scientific
Microtomy Brush	SLK1000	Cancer Diagnostics
Microtome	HM 315 / HM 325	Diagnostic Pathology
Fisherbrand™ Superfrost™ Plus Stain Slides	22-034979	Fisher Scientific
EZ-QUIK SLIDE STAINING RACK	NC0103846	Fisher Scientific
Tissue-Tek® Cold Plate	25608-942	VWR Scientific
Moist Mark Plus Slide/Cassette Marker	SKU: MP2100	Cancer Diagnostics
Dumont Forceps (Cover Slip Forceps)	11251-33	Fine Science Tools

NAME	CATALOG #	VENDOR
EMS Cover Glass Staining Racks	Catalog No.50-949-581	Fisher Scientific

SAFETY WARNINGS

- Use extreme care when working with microtome blades. They are extremely sharp.
- Use physical safety precautions when working with sharps (disposable blades).
- As these specimens are fixed in formaldehyde, gloves and other PPE are optional / personal preference.

BEFORE STARTING

- Ensure you have proper slides, blades, forceps, and your personal preference of gauzes/wipes.
- Use EXTREME CARE with the poly-llysine squares for mounting the CODEX tissue. Any nicks or dings in the mounting coverslip will make it immediately unusable for this process.
- Always use the specialized CODEX approved forceps to handle the poly-llysine square coverslips.

Microtome Preparation

1 Locate your tissue flotation bath / water bath and fill it completely with de-ionized or purified water. 5m

2 Turn the flotation bath on, and set the water temperature to 42°C. 5m

42 °C **Flotation Bath**

At this step, also inspect the microtome you are planning to use; Ensure it is clean, well maintained, and set at the correct angle.

Tissue Block Preparation

3

- The best practice for most all facing (trimming) and sectioning is to place a few paper towels or a Wypall on top of the cold plate tray, and dampen it with water.

Cold Plate Example: Sakura Tissue Tek Poly Cold Plate

- Ensure the tissue blocks you have chosen to section have been correctly embedded (1cm x 1cm), and all wax has been scraped/cleaned off the sides of the block.
- Ensure the tissue blocks have been correctly labeled with proper identification (case #, type, etc)

Coverslip Preparation

4 If the supply of the coated poly-l-lysine coverslips for CODEX microtomy is getting low, additional slips must be made ready. Please follow the protocol below if you are close to expiration on or running low of CODEX coverslips.

Franchesca Farris, Marda Jorgensen. Poly-Lysine Coverslip Preparation.
https://protocols.io/view/poly-lysine-coverslip-preparation-baeribd6

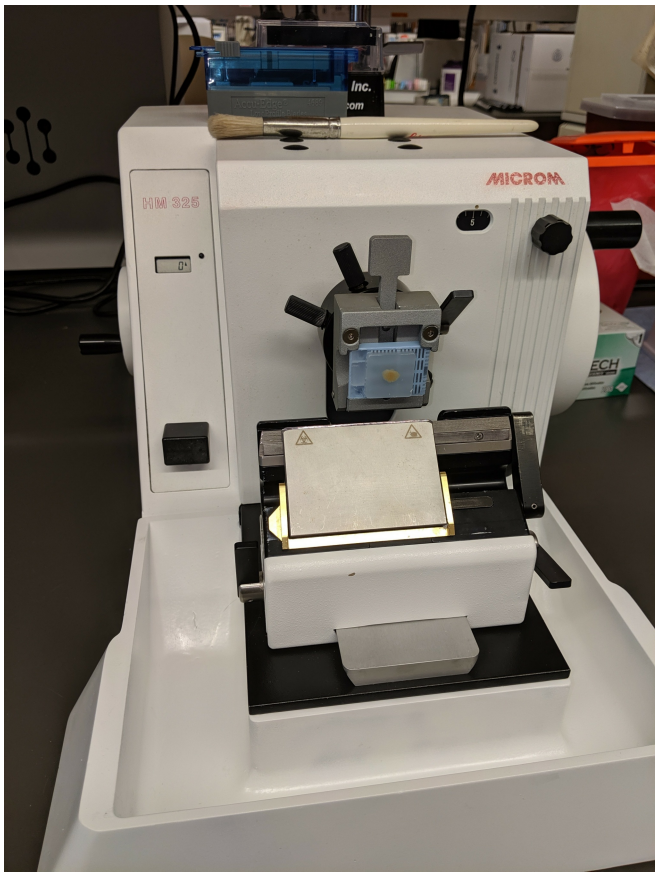
Tissue Block Facing

5 After locating the appropriate blades to use for your microtome, carefully select a blade from it's container, and place it inside the microtome's knife holder area. Be sure to secure it in place with the knife clamp/lock spanner. 10m

Microtome blade dispensing from storage box.

Before putting a microtome blade into the knife holder, BE SURE to utilize the locking lever under the flywheel.

6 After securing your knife and double checking your microtome settings one final time, retrieve a block from your ice tray and secure it in the microtome's chuck (block holder). 3m



A set up, tidy microtome, with a secured knife in the knife holder and tissue block in the microtome chuck.

7 After the block is secured, use the coarse advance wheel on the left side of the microtome to carefully, approach the block with the blade and cut a few thin sections to ensure the positioning is correct. Adjust if necessary.

8 ⚠

Notate all pertinent information on the [CODEX Microtomy Tracking Sheet](#) .

Cut Date	Logged Date	Tissue	Case #	Block ID	Trim Date	H/E Sections Collected	CODEX Sections Collected	Rack Location	CODEX Storage Location
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		
					☐ New ☐ Recut *µm removed:* ______	Thickness:______	Thickness:______		

Small view of the Codex Microtomy Sheet.

The process of sectioning CODEX specimens requires specific documentation at the tissue block trimming and microtomy stage.
As you begin to cut into a new or already used tissue block, you must keep track of how many microns of tissue are removed from the block during trimming, and also during microtomy.
Attached is a generic document that is used to keep record of what tissue block is being cut. All identification factors, trimming data, what tissue sections were used and their purpose, as well as storage information.
Formal documentation of how far into the tissue block you have traveled as well as how many sections were used is an extremely important and necessary function in this process.

9 Trim gently into the block to expose the tissue surface to a level where a complete representative section can be cut.

Trimming is normally done at a thickness of 10-30 µm. This can be performed with the advance flywheel alone, or a combination of both the advance and coarse advance wheels using the rocking method.

10 After each tissue block has been trimmed to expose a representative surface of the specimen, place each block back on your ice tray. Let each block chill for approximately 10-15 minutes.

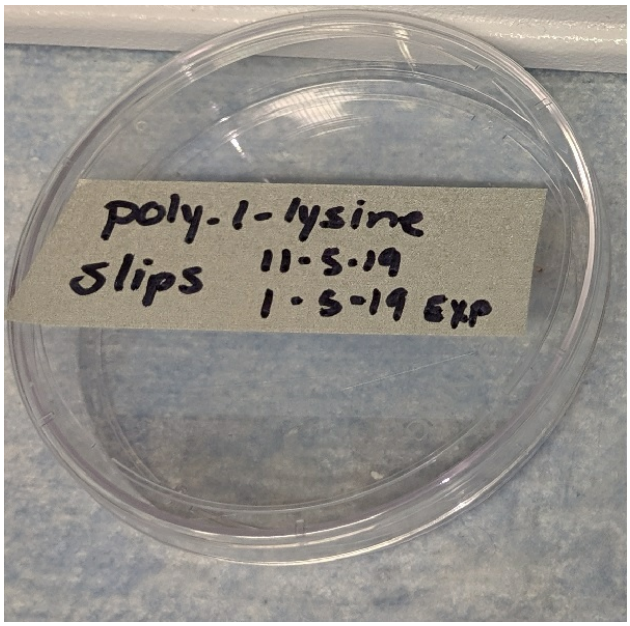
Cold wax allows thinner sections to be obtained by providing support for harder elements within the tissue specimen. The small amount of moisture that penetrates the block from the melting ice pieces or ice water will also make the tissue easier to cut.

Coverslip Preparation

11 10m

- Prepare a clean, dry surface near the microtome, and place your poly-l-lysine dish with the coverslips there.
- There is not a way to mark or identify these slips due to the nature of the processing on CODEX. Therefore, it is extremely important to pay attention and keep track of what you're cutting and where you place it in the special CODEX slide rack.

Wear gloves for this step to keep the tiny slips free of smudges, and to keep from contaminating ANY part of the container the slips are kept in.



poly-l-lysine coverslips secured in a sanitized petri dish with creation and expiration date.

12 Use an aerosol spray to clean coverslips from dust and lint prior to use.

Wear gloves for this step to keep the tiny slips free of smudges, and to keep from contaminating ANY part of coverslips or the container the slips are kept in.

Performing Microtomy

13 After your tissue blocks have chilled on the ice tray for 10 to 15 minutes, and are very cold to the touch, they should now be suitable for precision sectioning. 1h 30m

Remove your first block from the ice tray and secure it into the microtome's block chuck.

14 If you used the current exposed part of the microtome's blade on trimming, be sure to slide the knife over to a new, clean section before performing microtomy.

15 Using your coarse adjustment wheel on the left, adjust the block holder to be as close as possible to the edge of the knife, but not touching it.

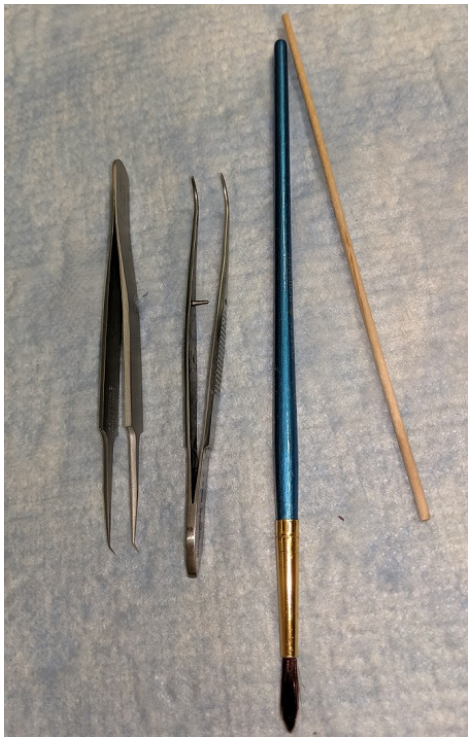
A helpful tip: When you adjust your chuck holder towards the blade and begin to see small water droplets from the condensation from the frozen block accumulate near the knife holder, stop using the coarse advance wheel.

16 Very carefully, unlock and rotate the hand wheel on the right side of the microtome so the block holder is moving up and down in a steady, even manner.
A ribbon of wax and tissue should begin to form down the front of the metal plate covering the knife holder.

- - Double check the micron thickness setting prior to this step. Normal thickness for CODEX sections is 5-10 µm.
- - It is normal to have to scrap the first few sections that appear on your ribbon due to holes or other cutting artifacts.

17 After your ribbon has begun to form on the front of the knife holder plate, use a pair of forceps (or whatever tool you prefer) to gently grip the bottom of the ribbon and another pair of forceps to gently grip the top of the ribbon nearest the blade.

Gently pull the ribbon up and away from the microtome and towards the waterbath.



A selection of tools ideal to use for securing and floating tissue ribbons during microtomy.

Lifting a ribbon of complete sections away from the knife holder plate.

via GIPHY

"Clip of sectioning a spleen by MJ @ University of Florida"

18 ▪ As carefully as possible, shift the ribbon of tissue sections toward your waterbath, and then quickly but gently lay the tissue out on the bath in a "dragging" type motion (either gently towards you, or gently away from you).

▪ Wait a few moments while the tissue sections sit on the waterbath, as the heated water will help expand any compression and remove some of the wrinkling or folding in the section.

Preparing to place a paraffin ribbon of tissue sections across a waterbath prior to creating a slide.

Small ribbon of tissue sections afloat on a waterbath - note they are very nice, with no wrinkling, folding, or knifelines.

via GIPHY

Small clip of moving a paraffin tissue ribbon to waterbath by MJ @ University of Florida

19 After your paraffin ribbon has floated on the waterbath for approximately 20-45 seconds, use your forceps or tool of choice to separate the sections from each other.

20 After selecting your section, use the specialty CODEX forceps and VERY carefully pick up the corner of the poly-l-lysine coverslip.

Dumont #5/45 - Cover Slip Forceps

21 VERY gently, dip the poly-l-lysine coverslip into the waterbath, and place adjacent to and slightly under your chosen section.

22 Adjust the section as desired on the slide, and gently lift upwards out of the waterbath.

via GIPHY

"Clip of a tech carefully selecting a poly-l-llysine coverslip and picking up a section for CODEX staining. Note the specialized staining rack."

23 Place your newly made slides/coverslips into the specialized metal staining rack and let your slides air dry overnight. **Following the air drying process, the sections must be stored at 4 °C for no more than a month, in a freezer-safe box where the sections cannot be stacked or damaged.** 24h

4 °C

Specialized custom staining rack appropriate for the poly-l-lysine coverslips.

As there is no way to mark these types of slides with identification, it is best to keep one case per rack / tray for organizational and accuracy purposes.

Cleanup Area 10m

24
- After you have sectioned your last tissue block, unlock the microtome knife blade holder's lever.
- Using a magnet or forceps, carefully remove the microtome blade and place into the waste container on the blade's box dispenser, or in a sharps container.

It is a good practice to clean your microtome if you are done for the day. This prevents paraffin gunk buildup on your microtome, waterbath and floor area, in addition to being ideal for safety.

25 Using a microtome brush, brush away all remnants of tissue and paraffin around your microtome, it's catch tray, chuck and behind the knife holder.

A commercially prepared product known as "ParaGuard" can assist with the removal of wax and provide a more efficient microtome clean up. It should be lightly used and vigilantly wiped off with gauze after use.

Example of Paraguard

26 Carefully remove the glass dish from your waterbath and dump the remaining water into your nearest sink.

27 Clean the emptied waterbath with warm water and dawn dish soap, then replace to the waterbath's bench unit.