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HuBMAP | Sectioning of FFPE Specimens

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Works for me

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

GE Research

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ABSTRACT

This method details sectioning of the FFPE HuBMAP specimens.

Protocol adopted from *Leigh Propper, Marda Jorgensen (12/13/2019). HuBMAP Tissue Sectioning for FFPE Specimens.* <https://dx.doi.org/10.17504/protocols.io.bahdib26>

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KEYWORDS

HuBMAP, sectioning, FFPE

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45539

A	B
Material	Catalog Number/Model
Water	In-house
Kimwipes	Fisher Scientific
Shandon™ Cartilage Curved Thumb Forceps	ThermoFisher (1631TS)
Flotation Bath	Boekel Scientific (145702)
Microtome Blades	Shandon (3153735)
4x4 Gauze	Fisher Scientific (1400249)
Charged Slides	SuperFrost Plus (12-550-15)
Staining Rack	Leica staining racks (045633919)
Marker	Stat Lab StatMark Lab pen (SMB-Bk)

SAFETY WARNINGS

Exercise extreme caution when working with microtome blades.

ABSTRACT

This method details sectioning of the FFPE HuBMAP specimens.

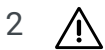
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BEFORE STARTING

- It is essential to read the complete instruction booklet before starting work.
- Users must be properly trained.
- Equipment and facility must be maintained and in good working order.

PREPARATION OF MICROTOME

- 1 Fill flotation bath with de-ionized or purified water. Set the water temperature to **42 °C**.

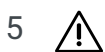


Inspect microtome to ensure it is clean, well-maintained, and set at the right angle.

PREPARATION OF TISSUE BLOCKS

- 3 Ensure the tissue blocks are correctly embedded and that all wax has been cleaned/scraped off the sides of the block. All tissue blocks should be labeled with appropriate ID.
- 4 Place dampened paper towels on top of the cold plate.

TISSUE BLOCK FACING



Be certain to utilize the locking level under the flywheel.

- 6 Carefully install the appropriate blade and place it inside the microtome's knife holder area.
- 7 Secure blade in place with knife clamp/lock spanner.
- 8 Select block from the ice tray and secure it in the chuck of the microtome (block holder).
- 9 Once block is secure, use the coarse advance wheel on the left-hand side of the microtome to carefully approach the block with the blade. Cut a few thin sections to ensure position is correct and adjust if necessary.
- 10 Trim gently (typically 10-30µm) into the block to expose a representative surface of the tissue. This can be done by advancing the fly wheel or by using a combination of the advance and coarse advance.
- 11 Repeat trimming for each block to be sectioned and place back into the ice tray when complete. Chill each block for 10-15 minutes.

PREPARATION OF SLIDES

- 12 Wear gloves to keep slides clean of debris and fingerprints.
- 13 Label each SuperFrost Plus slide with relevant ID information using a StatMark Lab pen.

SECTIONING

- 14 Remove the first block from the ice tray and secure it in the chuck of the microtome (block holder).
- 15 Adjust the block holder to be as close as possible to the edge of the knife, but not touching it, by using the coarse adjustment wheel on the left-hand side of the microtome.

A helpful tip from University of Florida: 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 Double-check micron thickness is set to $\pm 5 \mu\text{m}$.
- 17 Carefully unlock and rotate the hand wheel on the right-side of the microtome so that the block holder is moving up and down in a steady, even manner. A ribbon of wax should begin to form down the front of the metal plate covering the knife holder.

- 18 As the ribbon forms, use a pair of forceps to gently grip the bottom of the ribbon and another pair of forceps to gently grip the top of the ribbon nearest the blade, pulling the ribbon up and away from the microtome and toward the water bath.
- 19 Moving quickly, gently lay the ribboned sections out on the bath, using a dragging motion to prevent wrinkling/folding; allow 20-40 seconds for the warm water to help expand the ribbon and eliminate any wrinkling/folding.
- 20 Separate the first section you wish to keep using forceps.
- 21 Retrieve the appropriately labeled SuperFrost Plus slide.
 - 21.1 Dip the slide into the water bath, and place adjacent to and slightly under your chosen section.
 - 21.2 Adjust the section as desired on the slide with the forceps, and lift upwards out of the water bath.
- 22 Place slide into slide staining rack and allow to dry overnight.
- 23 Repeat for subsequent sections.

CLEAN-UP

- 24 Carefully remove the microtome blade and place into the waste container on the blade's box, or in a sharps container.
- 25 Brush away all remnants of tissue and paraffin around your microtome, its catch tray, chuck, and behind the knife holder.
- 26 Dump water from water bath and clean appropriately.