Nov 03, 2025

Tissue Sectioning for H&E - Pregnant Female Reproductive TMC

DOI
https://dx.doi.org/10.17504/protocols.io.eq2ly49kmlx9/v1

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Protocol Citation: Donald Pizzo, Mana Parast 2025. Tissue Sectioning for H&E - Pregnant Female Reproductive TMC. **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2ly49kmlx9/v1**

Protocol status: Working
We use this protocol and it's working

Created: September 30, 2025

Last Modified: November 03, 2025

Protocol Integer ID: 228623

Keywords: tissue sectioning, microtome, pregnant female reproductive tmc, protocol for microtome tissue, microtome tissue, animal tissue technology center, tissue, hematoxylin

Funders Acknowledgements:
Pregnant Female Reproductive Tissue Mapping Center
Grant ID: NIH U54 HD110347

Abstract

Here we describe the protocol for microtome tissue sectioning employed by the UC San Diego Human & Animal Tissue Technology Center prior to hematoxylin and eosin staining.

Materials

Distilled water
Leica microtome, model RM2265
Boekel Scientific Lighted Tissue floatation bath III; model 145702
Sakura Tissue-Tek Accu-Edge High profile microtome blades; catalog 4685-50
Microscope slides; Superfrost plus from Fisherbrand catalog 1255015
Microscope slide coverslip; #1.5 thick; 24 × 30 mm; catalog 48404-467
Kimtech Science Kimwipes Delicate Task Wipes, catalog 34120

Troubleshooting

Safety warnings

Microtomes can present a hazard when the sharp blades and foot treadles are uncovered. This sharps risk can also present a risk of exposure to specimens with blood borne pathogens. Protective gear such as cut-resistant gloves and eye protection, thorough training on the microtome instrument, and following universal precautions are advised.

Ethics statement

This protocol was used under the approval of the University of California San Diego Institutional Review Board. Users of this protocol for the purpose of sectioning human tissue should obtain prior approval from their Institutional Review Board (IRB) or equivalent ethics committee(s).

PROCEDURE

1 Fill water bath with distilled water and warm to 37ºC.

2 Label corresponding slide(s) for blocks to be cut.

3 Chill the tissue blocks: place blocks on a cold plate or ice tray with the tissue faced down.

4 Adjust/check that the section thickness is appropriate for the procedure/test. Unless otherwise specified, formalin-fixed paraffin-embedded blocks are commonly cut at a thickness of 5 µm to produce a wax ribbon, and H&E stained for histological evaluation.

5 Insert a microtome blade into the knife holder and secure in place. If the blade is not in use, have a knife guard in place.

6 If the block has not been previously sectioned, face the block at a thickness of 20 µm. Be cautious when facing smaller samples, or face the block using thinner sections (i.e. 5µm). Skip this step if the block was previously cut.

7 Clamp the block onto the block holder with the label on the left, then bring the block closer to the cutting edge of the blade with the "fine" or "gross" advance or retract controls/dials.

8 To begin sectioning: turn the rotary wheel clockwise. As the rotary wheel turns clockwise, the block will come in contact with the microtome blade. A ribbon of wax will form at the site of the blade holder. As the ribbon becomes longer, use an appropriate tool (forceps, brush, etc.) to hold the ribbon away from the microtome.

9 Place the wax ribbon onto the water bath by dropping the ribbon from one end to the other. Allow the sections to unwrinkle while floating on the water; a wrinkle-free ribbon should be obtained.

10 Use forceps or other suitable tool to separate the sections from the floating wax ribbon. Separate and capture sections one at a time.

11 Place a labeled slide at an angle at one edge of the wax section. Gently pull at the angle so the wax section will adhere to the slide in a wrinkle-free fashion.

12 Carefully shake off excess water (make sure the slide has firmly adhered before shaking).

13 Use a Kim wipe to skim the top of the water bath to clear debris and unwanted sections.

14 Place the slides in a slide holder or slide rack. Dry the slides as required for the staining procedure/test (air dried at room temperature, or at 42ºC or 60ºC in an oven).

15 After the last block has been cut, cover knife with the knife guard, and make sure the hand wheel is in the “LOCKED” position.

16 Discard blades into the appropriate sharps container and the wax trimmings in the waste container.