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Tissue Sectioning

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details formalin-fixed paraffin-embedded tissue sectioning on a microtome for subsequent tissue staining.

Attachments



[n2fcbpgrp.pdf](#)

161KB

Troubleshooting

Tissue Sectioning

2h 20m

- 1 Clean microtome blade with xylene (to remove paraffin) and then 100% ethanol (to dry).
 - 2 Mount blade on microtome, and tighten screws to hold it in place.
 - 3 Mount paraffin block in holder. Manually move the block near, but not touching the blade.
 - 4 Align the block to the blade in both left-right and top-bottom directions by iterative adjustments followed by checking the alignment.
 - 5 Once the block is directly apposed to the blade and aligned, switch cutting depth to $\pm 6 \mu\text{m}$.
 - 6 Hydrate block if needed with ammonia. Dry block after hydration.
 - 7 Begin sectioning the block. Grasp the first section lightly. Continue sectioning until you have a ribbon of sections (~6-10 sections).
 - 8 Use camel hair brush to lightly remove ribbon by brushing from one side to the other. Alternatively, lightly separate the section attached to the block from the remainder of the sections by scoring with a razor blade.
- ### Note
- This will allow you to keep a non-wrinkled section attached to the front of the next ribbon.
- 9 Transfer the ribbon to a 37°C water bath. Burst bubbles with a syringe needle. Score between sections with a razor blade.
 - 10 Gently spring apart sections with forceps and mount them in order on glass slides. Transfer the



slides to an upright rack to allow the water to wick off of the sections before transferring to a metal rack.

11 Continue sectioning (Steps 6-10) until the block is depleted of tissue.

12 Transfer slides to oven to dry at 42 °C for 02:00:00 or more or 60 °C for 00:20:00 or more.

2h 20m

