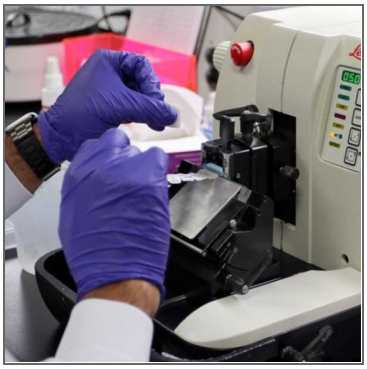

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Protocol status: Working
We use this protocol and it's working

Created: Sep 27, 2023

Buck Institute Morphology Microtome Protocol

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ABSTRACT

Buck Institute Morphology uses automatic rotary microtomes to section paraffin embedded tissue.
Safety considerations must be followed to safely acquire paraffin sections on an automatic microtome. The following protocol will describe steps to successfully and safely use the microtome for paraffin sections on glass slides.

MATERIALS

Morphology Microtome RM2255 -automatic rotary microtome
Morphology Microtome RM2155 -automatic rotary microtome
Epredia HM 355 S Microtome E System
Water Bath
Sakura AccuEdge 4689 Low Profile Blade

SAFETY WARNINGS

The microtome uses sharp disposable blades. Use caution when removing and installing blades. Always have the handwheel locked when manipulating the blade holder. Fully tighten the blade holder before attempting to section.

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PROTOCOL integer ID: 88497

Keywords: Micronomy, Microtome, Sections, paraffin blocks, histology, slide

Microtome setup

1 Turn on microtome, make sure all locks are set and blade guard is in place.

2 Have tools accessible - forceps, brushes, kimwipes, blades, ice water.
Place water bath near microtome. Water temperature 40-45 C

3 Set up blade holder angle between 5 to 10 degrees. Replace the blade if needed. Do not have the blade holder directly under specimen holder when installing the block. Orient yourself with each of
the clamps that hold the blade holder in position. Make sure they are tight (do not overtighten) before starting to section. It is dangerous to trim or sectionif the knife holder is not secure.

4 Set the sectioning thickness. Usual settings are 5-7 microns for sectioning, 10+ microns for trimming.

5 Paraffin blocks should be cold for optimum sectioning.

Install the paraffin block

6 Lock the handwheel before installing the paraffin block into the specimen holder. Open the clamp and install the block.

7 Orient the block so that block face travels parallel to the blade by using the adjustment screws on the specimen holder. This can be refined when the sample is close to the blade. This adjustment is to correctly position the block for optimal sectioning.

8 Carefully bring the block to the blade. You can manually rotate the handwheel or use the automatic functions. When the block touches the blade, you can slowly rough cut and adjust until an entire section is produced per rotation.

9 At this point you can trim the paraffin block to expose the sample.

Sectioning

10 Trim until you have the sample orientation correct and are getting complete sections per cycle.

10.1 Sections are cut gently with a uniform, slow rotation. Adjust your speed so that you can safely receive the sections.

10.2 Ribbons can be created with an even speed of rotation. You can use tweezers or brushes to help the ribbon stay down on the blade holder.

10.3 Between rotations some samples like liver require “soaking” Carefully, briefly place a damp Kim wipe on the sample before the rotation starts downward. Slow the speed to do this safely.

Water Bath section flotation

11 Bring your sections from the blade holder to the water bath and slowly lower your ribbon into the water. With a smooth motion you can avoid getting air bubbles or wrinkles.

12 Take a clean positively charged glass slide and slide it gently under the sections. Slowly raise it to connect the section to the slide, minimizing trapping air bubbles between sample and slide.

13 Collect the amount of sections required for each slide.

14 Drain the slide of water by placing vertically for several minutes

Remove blocks

14.1 After sectioning is completed, lock the flywheel and remove the block from the specimen holder.

15 Return the chuck holder to home position

16 If all sectioning has been completed, clean the equipment of wax. Remove disposable blade to used blade holder or sharps container.

17 Clean water bath if needed.