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Sectioning of Mouse Brain by Microtome

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Protocol status: Working

We use this protocol and it's working



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Abstract

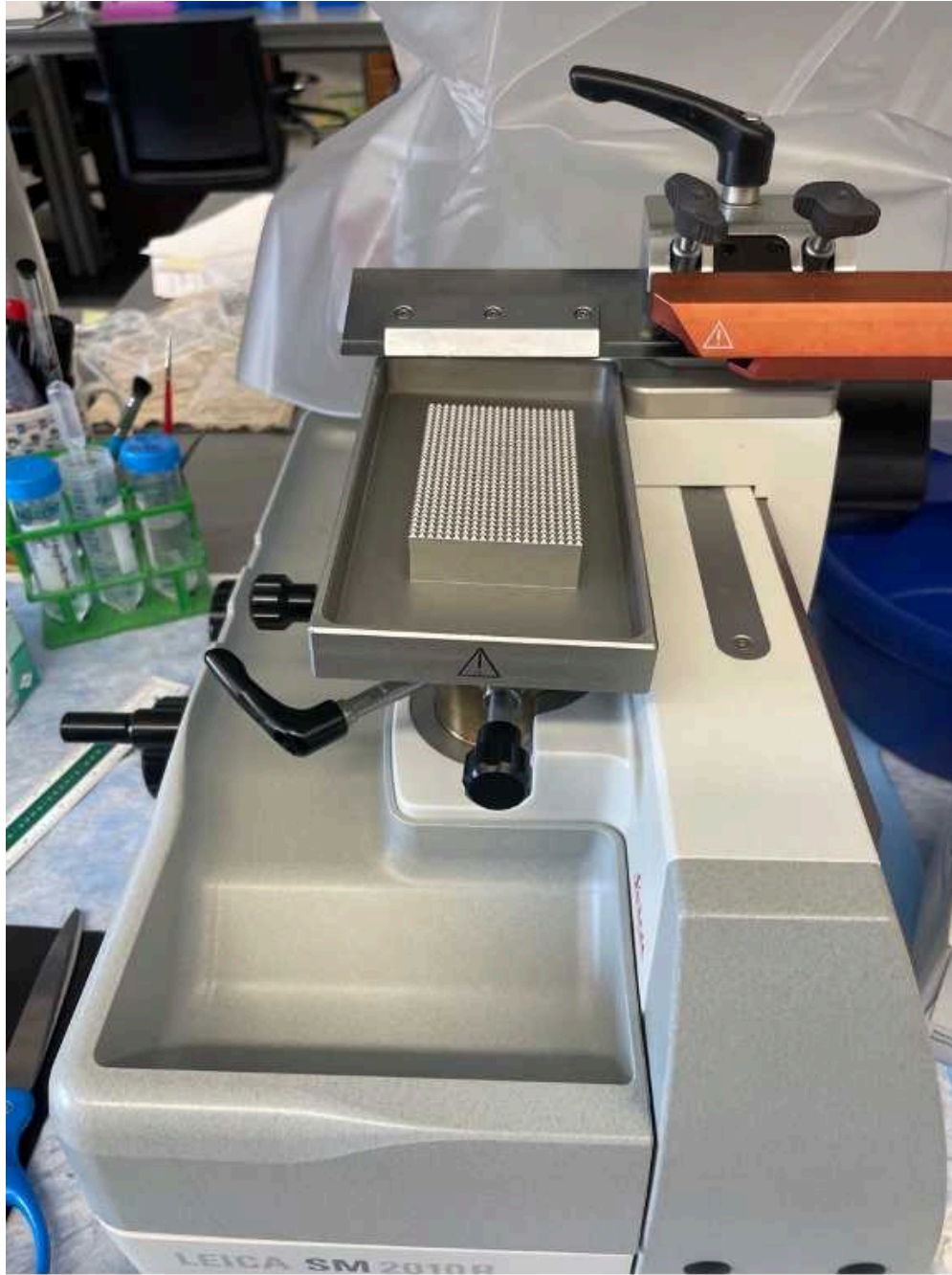
This protocol describes how to use the microtome to prepare and slice mouse brain sections for Immunohistochemistry

Troubleshooting



Preparation Methods

- 1 While preparing the microtome, make sure the blade is covered. The microtome used in the lab is from Leica Microsystems.
- 2 Slide the plate into the stage and secure it by tightening it with a screw.
- 3 Orient the plate so that it is centered.



Microtome setup, with the plate attached.

- 4 Add a few drops of 90% of ethanol on the sides of the plate and place dry ice to cool the microtome. The microtome will be cooled and ready for usage when the plate accumulates ice.
- 5 When the microtome is cooled, add a big drop of 1X Phosphate-Buffered solution [1X PBS] to the center of the plate
- 6 Quickly orient the brain on top of the 1X PBS bubble, ensuring that it is centered.



- 7 Double check the brain is in the center and straight. When it is, add more 1X PBS to secure it.
- 8 Add powdered dry ice over the brain to cool it. Let the brain cool for 2-5 minutes prior to slicing.
- 9 Prior to slicing, adjust the thickness setting. This knob is found on the side of the microtome.
- 10 Prepare the 24-well culture plate (Thermo Scientific, CAT # 142475] with cryoprotectant fluid. Brain sections will go here after they are sliced.

Slicing Methods

- 11 Remove the protective cover of the blade.
- 12 Move the stage up, placing the brain just below the blade.
- 13 Slowly move the blade towards you, slicing a section of the brain. To prevent the sections from folding and breaking, slice slowly and in a single continuous motion.
- 14 Gently scoop the brain section away from the blade, using a thin painting brush.
- 15 Slowly place the brush into cryoprotectant fluid. Gently move the brush to allow the section to unfold in the liquid.
- 16 Press the button on the side of the microtome to push the stage 1 point higher. This will allow you to slice the next brain section, at the thickness you have set.
- 17 Continue slicing until you have sliced the entirety of the brain.
- 18 Secure the 24-well plate with tape. Store brain sections in cryoprotected fluid at -20C.