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We use this protocol and it's working

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Sectioning of Mouse Brain by Cryostat

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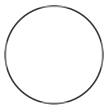

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ABSTRACT

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

Preparation Methods

1 After removing the brain from -80C storage, leave the brain on dry ice for 15-20 minutes. This will ensure there is not a big temperature difference when it is placed in the chamber of the cryostat. The cryostat my lab uses is Leica, model CM-30505.

2 Label microscope slides. For embryonic brains, you will need between 8 to 10 slides. For early post-natal brains, you will need up to 10 slides and for adult brains, you will need 13-15 slides.

3 Set the chamber temperature (CT) and object temperature (OT). My lab often works with -24C for CT and -21C for OT. The temperature will take a few minutes to set.

CT and OT Screen

4 When the temperatures of the cryostat are set, begin mounting the brain.

5 Place a disposable base mold [Fisher brand, CAT# 22-038-272] on the stand.

6 Fill half of the plate with embedding media

7 Gently transfer brain from dry ice to the center of the plate, securing it. Make sure to place it in the orientation you'd like.

8 Fill up the rest of the plate with embedding media. Note, when the embedding media freezes, it will turn white. This will make it difficult to tell the orientation of the brain. To prevent this, label the orientation of the brain on the plate.

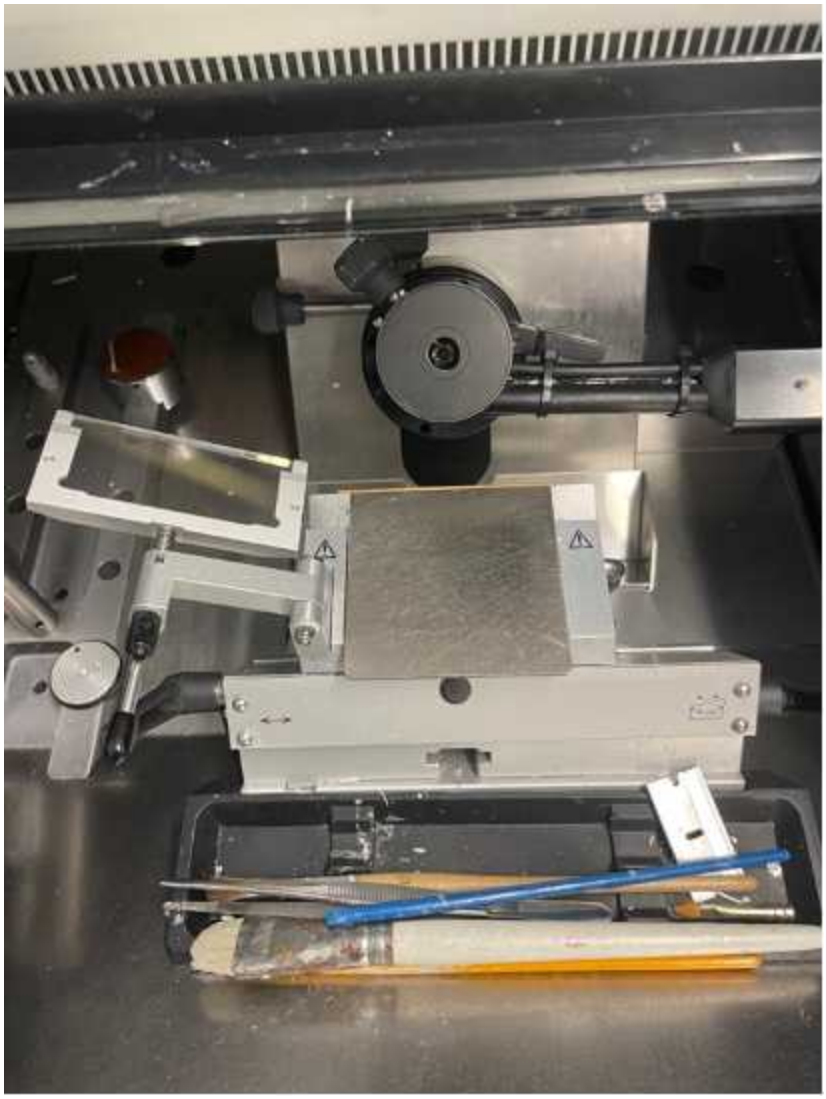
9 Place the plate under the cold press and press it to cool. Leave the press on the plate for 2-5 minutes.

10 Lift the cold press, releasing the plate

11 Remove the block from the plate. Now, you will have a frozen block.

12 Place the block on the concentric circle, and secure it with more embedding media

13 Attach the concentric circle to the stand, and secure it by tightening the screw above it



Setup inside of cryostat

14 Prior to slicing, double check the settings. Make sure you have the correct thickness

Slicing Methods

15 The cryostat can be run at an automated setting to section off the extra embedding media covering the brain. To do this, adjust the speed and thickness. Double press “RUN STOP” and “RUN ENABLE” to begin automated setting

Settings of Cryostat

16 Adjust the settings of the stand as needed to ensure that the blade is slicing full sections of the brain

17 Let the cryostat section the brain and occasionally stop it by double pressing “RUN STOP” and “RUN ENABLE” to brush off the embedding media on the stand

18 Stop the automated settings when you notice the cryostat has reached the brain. Begin manually collecting the brain sections.

19 Carefully mount each brain section onto a microscope slide by slightly tilting the slide over the brain section and pressing down. Be careful to not break the section, press down gently to lift the brain section.

20 Continue collecting sections until you have sliced the entirety of the brain

21 Store slides at -80C