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Brain Slicing for Immunohistochemistry

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ABSTRACT

Protocol is used for sectioning brains in order to use in downstream IHC experiments.

PROTOCOL CITATION

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GUIDELINES

This protocol is for perfused brains only. For snap-frozen brains, post-fix prior to using this protocol.

MATERIALS TEXT

- 1x PBS
- Petri dish
- Straight razor blade
- 1 50 ml falcon tube per brain
- Multiple 20 ml scintillation vials
- Paintbrush
- Mounting media
- 1x PBS + 0.1% sodium azide

BEFORE STARTING

Cryostat should be set to -9°C
Set the cutting thickness to 40 µm
Label vials and fill with 10-15 mL of PBS + Azide
Clean cryostat blade with 70% ethanol and set blade in cryostat for 30 minutes
Once the blade is locked into the cryostat, adjust the glass cover so that it very slightly extends pass the blade

1 Phosphate Buffered Saline **Thermo Fisher**

Wash brain with 1x **Scientific Catalog #28374**

1.1 Dispose of the current solution that brain is in into the proper waste stream

1.2 Transfer brain into 50 ml Falcon tube **Contributed by users** with **20-25 mL** of 1x PBS.
Invert the tube to wash and pour out PBS.

1.3 Repeat 2x for a total of 3 washes

2 Place cleaned brain flat onto petri dish. Using straight razor blade, cut of back half of cerebellum perpendicular to petri dish

3 Place the chuck onto dry ice. Place the brain in the center of the chuck with cerebellum on the bottom and use Richard-Allan Scientific™ Neg-50™ Frozen Section Medium, Colorless Neg-50 **Thermo Fisher Catalog #6502**
to add 1 layer of glue on bottom edge of brain.

3.1 Once the first layer is frozen, repeat process 2x for a total of 3 layers of mounting media.

4 Insert chuck into holder with dorsal side of brain facing up. The front of the brain should be pointing directly at you. This is to create coronal sections.

4.1 Start turning the handle slowly and make adjustments to the positioning of the brain if needed. Make sure each slice is cutting evenly at the correct angle.

5 Slice until you get to the region of interest. Place glass cover onto blade to collect the slices

6 Collect ~5 slices and use a wet paint brush to carefully transfer the slices in the first vial. If you need to collect more than 5 slices for this region, continue collecting in groups of 5 and transfer each group into the next vial.

7 Repeat steps 5-6 to get slices from all the regions of interests.

8 When finished, store with vials with the slices in **4 °C**