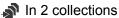


APR 15, 2024

## Histology Protocol



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#### **ABSTRACT**

This protocol details the histological processing of a mouse brain.





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Protocol status: Working

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PROTOCOL integer ID: 98231

**Keywords:** ASAPCRN

# 1d 7h 5m 1s **Histology Pro31hocol** 1 Prepare 4% paraformaldehyde in [M] 0.1 Molarity (M) PB, and Opt 7.4. Let chill to 4 °C. 2 Deeply anesthetize mouse with isofluorane until completely under (no toe pinch response, low to no breathing). 3 1s If the mouse has an electrode bundle implanted, quickly attach their tongue with an alligator clip to the negative port of a 9V battery, and touch each electrode connection for 600:00:01 with a wire attached to the positive port of the battery. This will create a small lesion for histological viewing of electrode placement. 4 Fix with a transcardial perfusion of $\bot$ 15 mL PBS followed by $\bot$ 50 mL of 4% PFA. 5 Overnight 8h Carefully remove the brain from the skull and post-fix in 🚨 50 mL of 4% PFA at 👢 4 °C 6 The next day, wash 3x with PBS, with a 15 min agitation in a cold room for the first wash, and 15 mins or longer agitation in a cold room for the remaining washes.

### protocols.io

7 15m The next day, wash with PBS, with a 00:15:00 agitation in a cold room for the first wash (1/3). The next day, wash with PBS, 00:15:00 or longer agitation in a cold room for the remaining washes 8 (2/3).9 The next day, wash with PBS, 00:15:00 or longer agitation in a cold room for the remaining washes (3/3).10 Embed the brains in 4% agarose. 11 Using a Leica VT1200S vibratome, slice the brain along the coronal axis at 4 50 undetermined thick slices. 12 If performing tyrosine hydroxylase immunostaining: 12.1 Place sections in a 24 well plate, 2-3 sections per well. 12.2 Wash 1x in PBS.

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Rinse slides with MilliQ, then coverslip with 🚨 155 µL of Vectashield Vibrance + DAPI mounting medium.

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Let set at Room temperature for at least 2 hours, and up to Overnight.

8h

16 When set, image slides on a VS200 slide scanner using the following protocol:

**16.1** Overview in brightfield.

**16.2** Focus in 10% Tritc (make sure focus points are not over the VTA).

**16.3** Image at:

- 1. 300ms 50% Cy5
- 2. 193ms 10% Tritc
- 3. 100ms 10% Fitc (only if GCaMP or TH present; otherwise, turn off this channel)
- 4. 20ms 10% DAPI