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Histology Protocol

In 2 collections

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ABSTRACT

This protocol details the histological processing of a mouse brain.

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Histology Pro31hocol

1d 7h 5m 1s

1 Prepare 4% paraformaldehyde in [M] 0.1 Molarity (M) PB, and pH 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 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 00:00:01 with a wire attached to the positive port of the battery. This will create a small lesion for histological viewing of electrode placement. 1s

4 Fix with a transcardial perfusion of 15 mL PBS followed by 50 mL of 4% PFA.

5 Carefully remove the brain from the skull and post-fix in 50 mL of 4% PFA at 4 °C Overnight. 8h

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.

7 The next day, wash with PBS, with a 00:15:00 agitation in a cold room for the first wash (1/3). 15m

8 The next day, wash with PBS, 00:15:00 or longer agitation in a cold room for the remaining washes (2/3). 15m

9 The next day, wash with PBS, 00:15:00 or longer agitation in a cold room for the remaining washes (3/3). 15m

10 Embed the brains in 4% agarose.

11 Using a Leica VT1200S vibratome, slice the brain along the coronal axis at 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.

12.3 Incubate for 02:00:00 in a blocking solution consisting of 5% goat serum, 3% bovine serum albumin, and 0.3% trition-x. 2h

12.4 Then transfer sections to a half-block solution containing 1:1000 rabbit α-TH, and leave Overnight at 4 °C on a shaker. 8h

12.5 Wash in [M] 0.1 Molarity (M) PBS with 0.1% tween.

12.6 Incubate for 04:00:00 in a half-block solution containing 1:1000 goat α-rabbit 488 . 4h

12.7 Wash in [M] 0.1 Molarity (M) PBS with 0.1% tween.

12.8 Wash in PBS.

13 Mount the sections onto SuperFrost glass slides. Let dry completely, then refresh with PBS for 00:20:00 . 20m

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

15 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