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Immunohistochemistry Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol has been approved by the California Institute of Technology's Institutional Animal Care and Use Committee (IACUC).

Brain Extraction

1 Mice were anesthetized with 150 uL pentobarbital (Euthasol), and their hearts were punctured.

2 Mice were perfused with ice-cold phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA).

3 Brains were extracted and preserved in 4% PFA for 48 hours while shaking at 4°C.

4 Brains were then transferred to PBS + 0.05% sodium azide for storage.

Preparation for Brain Slicing

5 To prepare PBS with 2% agarose and 0.02% sodium azide:

5.1 Suction filtered PBS.

5.2 Dissolved 10g of ultrafine agarose powder in 500 mL suction-filtered PBS.

5.3 Microwaved until agarose became transparent in the following intervals (30 seconds, 1 minute, 1 minute 30 seconds, 20 seconds).

5.4 Added 500 uL of 20% sodium azide to the agarose mixture.

5.5 Kept PBS in bead bath at 62°C to maintain in liquid phase.

6 To prepare PBS with 0.02% sodium azide:

6.1 Suction filtered PBS.

6.2 Added 500 uL of 20% sodium azide to 500 mL of suction-filtered PBS.

6.3 Added 1.5 mL of PBS with 0.02% sodium azide to each well of 12-well plate.

Brain Slicing

7 Whole brains were embedded in 2% agarose with 0.02% sodium azide by pouring agarose into a mold and letting it set in the 4°C fridge for 10 minutes.

8 Irregular borders of the agarose block were cut and removed, and blocks were fixed onto the vibratome stage using Gorilla super glue.

9 Embedded brains were sliced coronally into 50 µm sections using a vibratome at speed 8 and Personna razor blades.

10 Free-floating sections were placed in PBS + 0.02% sodium azide solution and stored at 4°C until staining.

10.1 Sections were placed in alternating wells of the 12 well plate.

Immunohistochemistry Preparation

11 Slices containing the striatum were identified and isolated using the Mouse Brain Atlas as a reference.

12 Slices were permeabilized for 30 minutes at room temperature in 0.5% Triton X-100, 3% BSA in PBS. For each well, 7.5 µL of Triton X-100 and 45 mg of BSA were added to 1.5 mL of filtered PBS. Scaling for 12 wells, 120 µL of Triton X-100 and 720 mg of BSA were added to 24 mL of filtered PBS.

13 Slices were washed with submerged for 5 minutes with PBS and washed 3 times.

14 Slices were blocked in 10% horse serum in PBS with 0.3% Triton X-100 and 0.04% sodium azide. To make the solution, 5 mL of horse serum was added to 138 µL of Triton X-100, 92 µL of 20% sodium azide, and 45 mL of PBS.

15 Slices were washed with submerged for 5 minutes with PBS and washed 3 times.

16 Slices were stained overnight at 4˚C while shaking with primary antibody TFAM Rabbit pAb (ABclonal Cat# A1926, RRID:AB_2763953) at a concentration of 1:200 in 0.1% Triton X-100, 3% BSA in PBS. To create the solution, 24 µL of Triton X-100 and 720 mg of BSA were added to 24 mL of PBS. To each well (1.5 mL), 7.5 µL of the primary antibody was added.

17 The following day, slices were washed with submerged for 5 minutes with PBS and washed 3 times.

18 Slices were stained while shaking with secondary antibody Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) (Abcam Cat# ab150075, RRID:AB_2752244) at 1:500 for 1 hour and 45 minutes in room temperature. To each well, 3 µL of the secondary antibody was added to 1.5 mL of blocking buffer (PBS with 0.3% Triton X-100 and 0.04% sodium azide).

19 Slices were washed with submerged for 5 minutes with PBS and washed 3 times.

19.1 In the final wash, 2 µL of DAPI (4',6-diamidino-2-phenylindole) was added to each well.

20 Slices were mounted on a glass cover slip using ProLong Diamond antifade mountant with 4',6-diamidino-2-phenylindole (DAPI).

Imaging and Analysis

21 Cover slips were stored at 4°C until imaging. Imaging was performed on a Zeiss LSM 900 microscope.

22 Images were taken with a 20x objective, with 4-6 pictures taken per animal.

23 ImageJ software was used to measure optical density in striatal region per animal.

24 Values were averaged per animal.