

Aug 09, 2024

Immunohistochemistry Protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg32y61v25/v1>

Livia Hecke Moraes¹, Jenny Ji¹

¹Caltech



Jenny Ji

Stanford

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg32y61v25/v1>

Protocol Citation: Livia Hecke Moraes, Jenny Ji 2024. Immunohistochemistry Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqdg32y61v25/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 07, 2024



Last Modified: August 09, 2024

Protocol Integer ID: 104920

Keywords: immunohistochemistry protocol protocol, immunohistochemistry, institutional animal care, iacuc, use committee, california institute, california institute of technology, protocol

Funders Acknowledgements:

ASAP

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 μ L 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 μ m 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 μ L of Triton X-100 and 45 mg of BSA were added to 1.5 mL of filtered PBS. Scaling for 12 wells, 120 μ L 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 μ L of Triton X-100, 92 μ L 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.