NOV 13, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gp356pvzp/v1

Protocol Citation: Ching-Chieh Chou, Judith Frydman 2023. Immunohistochemistry on free-floating and paraffin-embedded tissue sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp356pvzp/v1

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

Created: Nov 13, 2023

Immunohistochemistry on free-floating and paraffin-embedded tissue sections

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ABSTRACT

This protocol is used for free-floating frozen (30-50 microns) and paraffin-embedded (10 microns) tissue sections.

Last Modified: Nov 13, 2023

PROTOCOL integer ID: 90885

Keywords: ASAPCRN, Immunohistochemistry

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Materials

1 **Free-floating**

- PBS
- 0.3% Triton-X-100 in PBS 1x, stored at 4°C
- Normal Donkey Serum (NDS), stored at -20°C
- Blocking Buffer: 10% NDS and 0.03% Triton-X-100 in PBS 1x. Diluted from 100% NDS (stored at -20°C) and 0.3% Triton-X-100. Filtered with 0.22 µM filter.
- Primary Antibody
- Secondary Antibody
- Hoechst dye
- Mounting medium

Paraffin-embedded

- Deparaffinization station
- Slide rack
- Glass slide holder and tray
- Humidity and slide incubation chamber
- Xylene
- Ethanol
- Deionized water (diH2O)
- PBS
- Citrate buffer, pH 6.0, 10x
- 0.3% Triton-X-100 in PBS 1x, stored at 4°C
- Normal Donkey Serum (NDS), stored at -20°C
- Blocking Buffer: 10% NDS and 0.03% Triton-X-100 in PBS 1x. Diluted from 100% NDS (stored at -20°C) and 0.3% Triton-X-100. Filtered with 0.22 µM filter.
- Hydrophobic Barrier Pap Pen
- Primary Antibody
- Secondary Antibody
- Hoechst dye
- Mounting medium

Procedure for free-floating tissue staining

2 Collect free-floating frozen tissue sections and process in a 24-well culture plate.

2.1 **Day 1:** Wash the tissue for 3 X 5 min in PBS 1x.

2.2 Permeabilize the tissue in 0.3% Triton-X-100 in PBS 1x for 20 min at room temperature on a rocking shaker.

2.3 Wash the tissue for 3 X 5 min in PBS 1x.

2.4 Block the tissue in Blocking Buffer for 1 hr at room temperature on the rocking shaker.

2.5 Briefly rinse the tissue in PBS 1x once.

2.6 Prepare Primary Antibody in Blocking Buffer.

2.7 Incubate the tissue with Primary Antibody and transfer the plate to a refrigerator for overnight at 4°C.

2.8 **Day 2:** Wash the tissue for 3 X 5 min in PBS 1x.

2.9 Prepare Secondary Antibody in filtered 10% NDS in PBS 1x.

2.10 Incubate the tissue with Secondary Antibody for 1 hr at room temperature on the rocking shaker in the dark.

> **Note**
>
> After Secondary Antibody incubation, all procedures should be performed in the dark.

2.11 Wash the tissue for 2 X 5 min in PBS 1x.

2.12 Incubate the tissue in Hoechst (1:2000) in PBS 1x for 10 min at room temperature on the rocking shaker.

2.13 Rinse the tissue for 2 X 5 min in PBS 1x.

2.14 Transfer the tissue to a slide and remove solution around the tissue by Kimwipes.

2.15 Add mounting medium and put on a coverslip starting with top slowly to avoid generating any bubbles.

2.16 Gently move the coverslip and let the mounting medium cover the edge of the slide. Let it dry overnight in the dark.

2.17 **Day 3:** Acquire imaging by a microscope.

Procedure for paraffin-embedded tissue staining

3 Process paraffin-embedded tissue sections on glass slides.

3.1 **Day 1:** Place the slides in a slide rack and rehydrate sections by sequential washes in a deparaffinization station:

1. Xylene: 2 x 5 min
2. 100% Ethanol: 2 x 10 min
3. 95% Ethanol: 10 min
4. 70% Ethanol: 10 min
5. 50% Ethanol: 10 min
6. diH2O: 2 x 5 min

3.2 For antigen retrieval, dilute Citrate buffer in water to make 1x solution.

3.3 Fill a beaker with Citrate buffer 1x and place the beaker on a hot plate covered with foil. Boil the buffer to 100°C.

3.4 After the final step of rehydration, transfer the slides from the slide rack to a glass slide holder. Place the holder in the beaker and heat the slides for 20 min.

Note

Frequently monitor the temperature of Citrate buffer by a thermometer.

3.5 Take off the beaker from the hot plate and let it cool for 20 min.

3.6 Add PBS 1x to a glass tray. Put the glass slide holder in the tray and wash for 3 X 5 min on the rocking shaker.

3.7 After PBS wash, carefully remove all the solution around the section with Kimwipes and draw a circle around the tissue with PAP pen a few times.

3.8 Add Blocking Buffer (~200 µL) to cover the tissue without going over the PAP pen repellent border. Incubate for 2 hr at room temperature.

3.9 Prepare Primary Antibody in Blocking Buffer.

3.10 Remove all the Blocking Buffer around the tissue with Kimwipes.

3.11 Transfer the slides to a humidity and slide incubation chamber. Add water to bottom of incubation chamber.

3.12 Incubate the tissue with Primary Antibody (~200 µL) and transfer the chamber to cold room for overnight at 4°C.

3.13 **Day 2:** Knock the slides gently on Kimwipes and dry around the tissue.

3.14 Stagger slides in the glace slide tray and wash with PBS 1x for 3 X 5 min on the rocking shaker.

3.15 Prepare Secondary Antibody in 0.03% Triton-X-100 in PBS 1x.

3.16 After PBS wash, remove all the solution around the tissue with Kimwipes.

3.17 Transfer the slides to the humidity and slide incubation chamber. Add water to bottom of incubation chamber.

3.18 Incubate the tissue with Secondary Antibody (~200 µL) for 1 hr at room temperature in the dark.

> **Note**
>
> After Secondary Antibody incubation, all procedures should be performed in the dark.

3.19 Gently wipe off the solution around the tissue. Stagger slides in the glace slide tray and wash with PBS 1x for 2 X 5 min on the rocking shaker, covered with foil.

3.20 Remove solution around the tissue. Transfer the slides to the humidity and slide incubation chamber.

3.21 Incubate with Hoechst dye at 1:2000 dilution in PBS 1x for 10 min.

3.22 Place the slides in the glace slide tray and rinse with PBS 1x for 2 X 5 min on the rocking shaker.

3.23 After the final wash, gently tap on the slides and remove all the solution around the tissue. Dry the slides onvernight in the dark.

3.24 **Day 3:** Add mounting medium on the coverslip. Place the slides on the coverslip slowly to avoid generating any bubbles.

3.25 Flip the slides with coverslip on the top. Gently move the coverslip and let the mounting medium cover the edge of the slide.

3.26 Dry the slide overnight in the dark.

3.27 **Day 4:** Acquire imaging by a microscope.