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Immunohistochemistry of free floating slices

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We use this protocol and it's working

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Disclaimer

ETHICS DISCLAIMER
The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

This protocol details the immunohistochemistry of free floating slices.

Steps

2w 0d 5h 40m

1

2w

Note

Step 1 is for fresh tissue only, otherwise, start directly to step 2

Fix with paraformaldehyde (PFA) 4% Overnight at Room temperature .
- 4 g of PFA
- Dissolve in 100 mL de PBS (pH 7.3) previously heated to around 60 °C .
- Mix the solution until it completely clears up.
- Filter and store at 4 °C for a duration of 336:00:00 .

2 Rinse with PBS 1X (3X 10 min) under agitation.

2.1 Rinse with PBS 1X for 00:10:00 under agitation (1/3). 10m

2.2 Rinse with PBS 1X for 00:10:00 under agitation (2/3). 10m

2.3 Rinse with PBS 1X for 00:10:00 under agitation (3/3). 10m

3 Permeabilize and block the non-specific primary antibodies bindings in a 24-well multiwell plate
by incubating under agitation for 01:00:00 with 0.5 mL to 1 mL of a BSA enriched ab solution (10 mL of ab solution for 1 g of BSA). 1h

4 All the subsequent steps are done using the following ab solution:

- 95 mL of PBS 7.3
- 20 mg of NaN3 (final 0.02 %)
- 300 µL of Triton X-100 (final 0.3 %)

- 500 mg of BSA (final 0.5 %)
- 5 mL of goat serum (final 5 %)

5 Dilute the primary antibodies in the ab solution and apply 0.5 mL per well (up to 10 slices) Overnight at Room temperature under agitation. 1h

6 Rinse the slices with PBS (3X 10 min) under agitation.

6.1 Rinse the slices with PBS for 00:10:00 under agitation (1/3). 10m

6.2 Rinse the slices with PBS for 00:10:00 under agitation (2/3). 10m

6.3 Rinse the slices with PBS for 00:10:00 under agitation (3/3). 10m

7 Dilute the secondary antibodies in the ab solution and apply 0.5 mL per well (up to 10 slices) for 02:00:00 at Room temperature under agitation. 2h

8 Rinse the slices with PBS (3X 10 min) under agitation.

8.1 Rinse the slices with PBS for 00:10:00 under agitation (1/3). 10m

8.2 Rinse the slices with PBS for 00:10:00 under agitation (2/3). 10m

8.3 Rinse the slices with PBS for 00:10:00 under agitation (3/3). 10m

9 Mount the slices on charged (+) glass slides submerged in PBS.

10 Let the mounted slices dry (about 00:10:00). 10m

Note

*Don’t wait too long otherwise salt deposits will start to appear and affect your staining.

11 Seal the slides with 100 µL - 120 µL /slide of Fluoromount G and a rectangular glass coverslip and seal the coverslip to the slide with nail polish.