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

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Immunofluorescence staining, vibratome sections

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ABSTRACT

Protocol for performing immunofluorescence staining on free-floating vibratome cut brain sections from rats or mice.

MATERIALS

Antigen retrieval solution (10 mM citrate buffer pH 6.0)

Prepare stock solutions:
Solution A : 0.1 M citric acid solution
Solution B : 0.1 M sodium citrate solution

Prepare working solution:
Add 9 mL of Stock solution A and 41 mL of Stock solution B to 400 mL of AD
Adjust pH to 6.0
Fill until 500 mL with AD

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ASAP (Aligning Science Across Parkinson's)

Day 1

2h

1 Transfer sections to be stained to a new 24-well plate filled with 1X PBS. All incubations are with 500 µL solution per well unless stated otherwise.

2 1X PBS rinse.

3 Antigen retrieval step (using oven).

3.1 Incubate sections in antigen retrieval solution (10 mM citrate buffer pH 6.0 ; see recipe in Materials) at 80^{o}C for 00:30:00. 30m

3.2 Place the well plate in the same buffer on ice for 00:20:00. 20m

4 1X PBS rinse.

5 Wash sections in 1X PBS for 00:05:00 at room temperature on wobbler. 5m

6 Wash sections in 1X PBS for 00:05:00 at room temperature on wobbler. 5m

7 Incubate sections with 250 µL blocking solution (PBS-T + 10% donkey serum) per well for 01:00:00 at room temperature on wobbler. (PBS-T = PBS + 0.1% Tergitol) 1h

8 Dilute primary antibodies at the required concentration in PBS-T + 10% donkey serum.

9 Incubate sections with 250 µL primary antibodies overnight at room temperature.

Day 2 2h

10 1X PBS-T rinse.

11 Wash sections in 1X PBS-T for 00:05:00 at room temperature on wobbler. 5m

12 Wash sections in 1X PBS-T for 00:05:00 at room temperature on wobbler. 5m

13 Dilute secondary antibodies at the required concentration in PBS-T.

14 Incubate sections with 250 µL secondary antibodies for 02:00:00 at room temperature (in the dark). 2h

15 1X PBS rinse.

16 Wash sections in 1X PBS for 00:05:00 at room temperature on wobbler. 5m

17 Wash sections in 1X PBS for 00:05:00 at room temperature on wobbler. 5m

18 Briefly rinse sections in 1/2 PBS + 1/2 AD and allow to dry.

19 Mount with Mowiol.