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Immunofluorescence staining, vibratome sections

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Protocol status: Working

We use this protocol and it's working

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Funders Acknowledgements:

ASAP (Aligning Science Across Parkinson's)

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



Day 1

2h

- 1 Transfer sections to be stained to a new 24-well plate filled with 1X PBS. All incubations are with  500 μ L 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°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 μ L 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 μ L 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 μ L 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.