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Immunofluorescence staining ASE, vibratome sections

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Protocol status: Working

We use this protocol and it's working

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ASAP (Aligning Science Across Parkinson's)

Abstract

Protocol for performing immunofluorescence staining using antibody signal enhancement (ASE) on vibratome cut brain sections from rats or mice.

Materials

Antigen retrieval solutions

Tris-HCl-EDTA buffer pH 9.0 (25mM Tris)

- Tris-HCl 1.97 g
- EDTA 0.2 g
- Distilled water 500 mL
- Mix to dissolve. Adjust pH to 9.0

Tris-HCl-EDTA buffer pH 9.0 (25mM Tris) + 0.05% SDS

- 95 mL Tris-HCl-EDTA buffer (25mM Tris)
- 5 mL SDS 1%
- Mix to dissolve.

ASE staining solutions

ASE wash buffer (PBS + 0.5% Tween-20)

- 500 mL PBS
- 2.5 mL Tween-20
- Mix to dissolve.

ASE blocking solution - 2 mL (2% donkey serum + 50 mM glycine + 0.05% Tween-20 + 0.1% Tergitol + 0.1% BSA)

- 1 mL 100 mM glycine
- 0.2 mL BSA 1%
- 0.8 mL PBS
- 1 μ L Tween-20
- 2 μ L Tergitol
- 40 μ L donkey serum
- Mix to dissolve.

ASE primary antibody buffer - 2 mL (10 mM glycine + 0.05% Tween-20 + 0.1% Tergitol + 0.1% H₂O₂)

- 1.8 mL PBS
- 0.2 mL 100 mM glycine
- 1 μ L Tween-20
- 2 μ L Tergitol
- 6.6 μ L 30% H₂O₂
- Mix to dissolve.

ASE secondary antibody buffer - 2 mL (0.1% Tween-20)

- 2 mL PBS
- 2 μ L Tween-20



- Mix to dissolve.

1% BSA stock

- 0.1 g BSA
- 10 mL PBS
- Mix to dissolve.

100 mM glycine stock

- 0.05 g glycine
- 6.6 mL PBS
- Mix to dissolve.



Day 1

- 1 Briefly rinse sections in 1/2 PBS + 1/2 AD and mount on Superfrost Plus Slides.
- 2 Air dry slides overnight at room temperature.

Day 2

3h 57m

- 3 1X PBS rinse.
- 4 Note: All washes performed in Tissue-Tek Staining Trays, on the wobbler. 100 mL / tray.
- 5 Antigen retrieval step (using Steamer).
 - 5.1 Fill water to the maximum level in the steamer (use distilled water).
 - 5.2 Place Tissue-Tek Staining Trays containing Antigen retrieval solution (Tris-HCl-EDTA buffer pH 9.0 + 0.05% SDS ; see recipe in Materials) in the steam bowl. Fill with 100 mL / tray.
 - 5.3 Wait at least  00:15:00 for the solutions in the steam bowl to reach 95-98°C. 15m
 - 5.4 Place the glass slides with the tissue in the Antigen retrieval solution.
 - 5.5 Start timer for antigen retrieval ( 00:30:00). 30m
 - 5.6 Refill with distilled water the tank as needed.



- 6 After antigen retrieval : Place the Tissue-Tek Staining Trays from the Steamer on ice for  00:20:00 (in cold room). 20m
- 7 Dry slides and add hydrophobic barriers on each side of the tissue section. Do not let the barrier touch the tissue.
- 8 Wash slides with ASE Wash buffer (PBS + 0.5% Tween-20 ; see recipe in Materials) for  00:03:00 at room temperature on wobbler. 3m
- 9 Wash slides with ASE Wash buffer for  00:03:00 at room temperature on wobbler. 3m
- 10 Prepare box for slide incubation. Place wet tissue paper on the bottom to create a humid chamber. Add the glass slides inside facing up.
- 11 Pipette  500 μL ASE Blocking solution (PBS + 2% donkey serum , 50 mM glycine, 0.05% Tween-20, 0.1% Tergitol, 0.1% BSA ; see recipe in Materials) per glass slide.
- 12 Block for  00:30:00 at room temperature. 30m
- 13 Discard the blocking solution, and pipette instead  500 μL of primary antibody solution per glass slide. Dilute primary antibodies in ASE primary antibody buffer (PBS + 10 mM glycine, 0.05% Tween-20, 0.1% Tergitol, 0.1% H_2O_2 ; see recipe in Materials).
- 14 Incubate with primary antibodies overnight at 4°C.

Day 3

3h 57m

- 15 Wash slides with ASE wash buffer (quick rinse).
- 16 Wash slides with ASE wash buffer for  00:03:00 at room temperature on wobbler. 3m



- 17 Wash slides with ASE wash buffer for 00:03:00 at room temperature on wobbler. 3m
- 18 Place the glass slides in the incubation box. Pipette 500 μL of secondary antibody solution per glass slide. Dilute secondary antibodies in ASE secondary antibody buffer (PBS + 0.1% Tween-20 ; see recipe in Materials).
- 19 Incubate with secondary antibodies in the dark for 02:00:00 at room temperature. 2h
- 20 Wash slides with PBS (quick rinse).
- 21 Wash slides with PBS for 00:05:00 at room temperature on wobbler. 5m
- 22 Wash slides with PBS for 00:05:00 at room temperature on wobbler. 5m
- 23 Remove hydrophobic barriers. Allow sections to dry and mount with Mowiol.