FEB 16, 2024

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj37zplk5/v1

Protocol Citation: eduard.bentea, María Sanchiz Calvo, Veerle Baekelandt 2024. Immunofluorescence staining ASE, vibratome sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj37zplk5/v1

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

Immunofluorescence staining ASE, vibratome sections

eduard.bentea[1], María Sanchiz Calvo[1], Veerle Baekelandt[1]

[1]Katholieke Universiteit Leuven

eduard. eduard.bentea

ABSTRACT

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

Created: Feb 16, 2024

Last Modified: Feb 16, 2024

PROTOCOL integer ID: 95339

Keywords: ASAPCRN

Funders Acknowledgement:
ASAP (Aligning Science Across Parkinson's)

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 µL Tween-20
- 2 µL Tergitol
- 40 µL donkey serum
- Mix to dissolve.

ASE primary antibody buffer - 2 mL (10 mM glycine + 0.05% Tween-20 + 0.1% Tergitol + 0.1% H_2O_2)

- 1.8 mL PBS
- 0.2 mL 100 mM glycine
- 1 µL Tween-20
- 2 µL Tergitol
- 6.6 µL 30% H_2O_2
- Mix to dissolve.

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

- 2 mL PBS
- 2 µL 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^{o}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^{O}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.