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🌐 Immunofluorescence staining protocol with Antigen Retrieval

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ABSTRACT

This protocol details the immunofluorescence staining protocol with antigen retrieval.

MATERIALS

⊗ ProLong[®]; Diamond Antifade Mountant **Thermo Fisher Catalog #P36961**

⊗ Universal HIER antigen retrieval reagent (10X) **Abcam Catalog #ab208572**

OPEN  ACCESS



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

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PROTOCOL integer ID: 97924

Keywords: ASAPCRN

Day 1

1 Staining protocol for $\pm 35 \mu\text{m}$ free floating mouse brain sections.

2 Wash tissue sections 3 times (5 minutes each wash) in PBS to remove cryoprotectant solution.



2.1 Wash tissue sections for 00:05:00 in PBS to remove cryoprotectant solution (1/3).

5m

2.2 Wash tissue sections for 00:05:00 in PBS to remove cryoprotectant solution (2/3).

5m

2.3 Wash tissue sections for 00:05:00 in PBS to remove cryoprotectant solution (3/3).

5m

3 Mount tissue onto positively charged slides.

4 Allow slides to air dry then place in oven set at 37 °C - 50 °C Overnight . 5m



Note

Can use slide warmer or incubator for this step.

Day 2

5 If you would like to use a PAP pen to draw a box around your tissue sections, do that now and wait for slides to dry.

6 Wash slides in PBS for 00:05:00 . 5m



7 Preheat steamer with a Coplin jar containing the antigen retrieval buffer until temperature reaches 95 °C - 100 °C (about 00:10:00 10m

Note


This protocol uses Universal HIER antigen reagent as the buffer. This comes at 10X reagent, therefore, you need to dilute 1:10 with distilled water for 1x concentration of the desired volume.

8 Immerse slides into the preheated buffer in the steamer for 00:30:00 . 30m


9 After 30 minutes turn off the steamer but allow to cool to Room temperature (about 20- 00:30:00 30m
) before progressing to the next step.

10 Wash slides in PBS 0.1% Triton X-100 (PBST) 2 x 2 min.



10.1 Wash slides in PBS 0.1% Triton X-100 (PBST) for  00:02:00 (1/2).

2m

10.2 Wash slides in PBS 0.1% Triton X-100 (PBST) for  00:02:00 (2/2).

2m

11 Wash slides in PBS 3 x 5 min.



11.1 Wash slides in PBS for  00:05:00 (1/3).



5m

11.2 Wash slides in PBS for  00:05:00 (2/3).

5m


11.3 Wash slides in PBS for  00:05:00 (3/3).



5m




12 Block for  02:00:00 at  Room temperature in PBS (0.4% BSA, 10% Goat normal serum, 0.3% triton X-100).

2h

Note

- Move slides to black slide box for this step and subsequent steps.
- Use 1-2ml solution for washes and  700 µL for antibody incubations.

13 Incubate  Overnight with primary antibody in PBS (2% Goat normal serum, 0.3% triton X-100)  20m

  Overnight at  Room temperature .



Day 3

9h




14 Wash slides in PBST 3 x 10 min.






14.1 Wash slides in PBST for  00:10:00 (1/3). 

14.2 Wash slides in PBST for  00:10:00 (2/3). 

14.3 Wash slides in PBST for  00:10:00 (3/3). 

15 Incubate slides with secondary antibody in PBS for  04:00:00 at  Room temperature or at  8h

  4 °C  Overnight .



16 Wash slides in PBST 3 x 10 min.



16.1 Wash slides in PBST for 00:10:00 (1/3).

10m

16.2 Wash slides in PBST for 00:10:00 (2/3).

10m

16.3 Wash slides in PBST for 00:10:00 (3/3).

10m

17 Incubate slides with Hoescht stain (1:5000) for 00:30:00 at Room temperature .

30m



18 Wash slides in PBST 3 x 10 min.



18.1 Wash slides in PBST for 00:10:00 (1/3).

10m


18.2 Wash slides in PBST for 00:10:00 (2/3).

10m

18.3 Wash slides in PBST for  00:10:00 (3/3).


10m

19 Mount slides using ProLong™ Diamond Antifade Mountant.

20 Dry slides on a flat surface at  Room temperature in the dark.

Note

Put them under aluminum foil or a box on the bench.

21 Store slides at  4 °C the next day.