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# Immunofluorescence staining protocol with Antigen Retrieval

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

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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

## Troubleshooting

## Day 1

1 Staining protocol for 35 µm 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 ) before progressing to the next step. 30m

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

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

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

11 Wash slides in PBS 3 × 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) Overnight at Room temperature . 20m

## Day 3 9h

14 Wash slides in PBST 3 × 10 min.

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

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

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

15 Incubate slides with secondary antibody in PBS for 04:00:00 at Room temperature or at 4 °C Overnight . 8h

16 Wash slides in PBST 3 × 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
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18 Wash slides in PBST 3 × 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.