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Immunofluorescence staining of cultured mouse hippocampal neurons

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We use this protocol and it's working

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Abstract

This protocol describes the immunofluorescence staining of cultured neurons.

Materials

Reagents

- 4% Paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4
- PBS (phosphate-buffered saline)
- Normal goat serum (NGS) (G9023 Sigma-Aldrich)
- Triton X-100 (BP151, Fisher Scientific)
- Primary antibodies (user-defined)
- Secondary antibodies (Alexa Fluor series or others)
- ProLong Gold Antifade Mountant (P36930, Thermo Fisher Scientific)

Consumables

- Glass coverslips
- Culture dishes

Equipment

- Nikon Ti microscope, CSU-W1 spinning disk confocal unit, Andor Zyla 4.2 sCMOS camera
- 60× oil immersion objective (PlanApo, NA 1.40)
- Micro-Manager software (v2.0-gamma)
- ImageJ / Fiji (NIH)

Fixation

1 Remove culture medium and rinse coverslips briefly with PBS.

2 Fix neurons with 4% PFA in 0.1 M phosphate buffer for 10–15 min at room temperature.

3 Wash coverslips 3 times with PBS.

Permeabilization and Blocking

4 Incubate coverslips for 15–30 min in PBS containing: 4% NGS, 0.1% Triton X-100

Primary Antibody Incubation

5 Prepare primary antibodies in PBS containing: 1% NGS, 0.025% Triton X-100

6 Incubate coverslips overnight at 4°C in a humidified chamber.

7 Wash coverslips 3 times with PBS.

Secondary Antibody Incubation

8 Prepare secondary antibodies in PBS containing: 1% NGS, 0.025% Triton X-100
Incubate coverslips for 1 h at room temperature, protected from light.

9 Wash coverslips 3 times with PBS, protecting from light.

Mounting

10 Mount coverslips on slides using ProLong Gold Antifade Mountant.

Imaging

11 Visualize fluorescence using a Nikon Ti + CSU-W1 spinning disk confocal system equipped with a 60× oil- immersion (NA 1.4) objective.

12 Process images using ImageJ (NIH).