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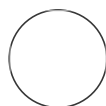
Protocol status: Working

Immunofluorescence

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ABSTRACT

This protocol details methods for the immunofluorescence staining of neurons.

ATTACHMENTS

[326 - 698.pdf](#)

MATERIALS

Solutions to prepare

Tyrode solution:

A	B
136 mM	NaCl
2.5 mM	KCl
2 mM	CaCl ₂
1.3 mM	MgCl ₂
10 mM	HEPES
10 mM	glucose

Fixative solution:

4% Paraformaldehyde solution (PFA) (Electron Microscopy Sciences, #15710) in 4% sucrose-containing **1M 0.1 Molarity (M)** PB buffer (**pH 7.3**).

Blocking and permeabilization buffer (called blocking buffer)

- 3% BSA (to quench non-specific protein binding sites)
- 0.2% Triton X-100 (to permeabilize cells) in PBS

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Immunofluorescence, staining,
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Protocol

2h 50m

- 1 Wash cultured hippocampal neurons with pre-warmed tyrode.



- 1.1 Wash cultured hippocampal neurons with pre-warmed tyrode. (1/3)



- 1.2 Wash cultured hippocampal neurons with pre-warmed tyrode. (2/3)



- 1.3 Wash cultured hippocampal neurons with pre-warmed tyrode. (3/3)



- 2 Fix the cells with fixative solution for  00:15:00 at  Room temperature

15m

- 3 After fixation, wash the cells with PBS.



3.1 Wash the cells with PBS. (1/3)



3.2 Wash the cells with PBS. (2/3)



3.3 Wash the cells with PBS. (3/3)



4 Incubate with blocking buffer for 00:30:00 at Room temperature

30m



5 Wash the cells briefly with PBS and incubate the cells with primary antibodies (1:500 ~ 1:2000) in a blocking buffer for 01:00:00 at Room temperature on a rocking platform.

1h



6 Wash with PBS on a rocking platform.



6.1 Wash with PBS for 00:02:00 on a rocking platform. (1/5)


2m



6.2 Wash with PBS for 00:02:00 on a rocking platform. (2/5)

2m



6.3 Wash with PBS for  00:02:00 on a rocking platform. (3/5)


2m



6.4 Wash with PBS for  00:02:00 on a rocking platform. (4/5)



2m



6.5 Wash with PBS for  00:02:00 on a rocking platform. (5/5)

2m



7 Incubate the cells with secondary antibodies (for example Alexa-fluor-labeled) (1:1000) in a blocking buffer for  00:45:00 on a rocking platform at  Room temperature in the dark.

45m



8 Decant the secondary antibodies and wash with PBS.

8.1 Wash with PBS for  00:02:00 each in the dark. (1/5)

2m



8.2 Wash with PBS for  00:02:00 each in the dark. (2/5)

2m



8.3 Wash with PBS for  00:02:00 each in the dark. (3/5)

2m



8.4 Wash with PBS for  00:02:00 each in the dark. (4/5)

2m



8.5 Wash with PBS for  00:02:00 each in the dark. (5/5)

2m



9 Observe the fluorescence signal using an inverted confocal microscope or mount the samples with Prolong Gold antifade reagent for long-term storage

