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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	CaCl2
	1.3 mM	MgCl2
	10 mM	HEPES
	10 mM	glucose

Fixative solution:
4% Paraformaldehyde solution (PFA) (Electron Microscopy Sciences, #15710) in 4% sucrose-containing [M] 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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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