

Immunofluorescence

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Abstract

This protocol details methods for the immunofluorescence staining of neurons.

Solutions to prepare

1. Tyrode solution

136 mM NaCl, 2.5 mM KCl, 2 mM CaCl₂, 1.3 mM MgCl₂, 10 mM HEPES and 10 mM glucose.

2. Fixative solution

4% Paraformaldehyde solution (PFA) (Electron Microscopy Sciences, #15710) in 4% sucrose-containing 0.1M PB buffer (pH7.3).

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

Protocol

1. Wash cultured hippocampal neurons with pre-warmed tyrode (2-3 times)
2. Fix the cells with fixative solution for 15 minutes at room temperature.
3. After fixation, wash the cells three times with PBS and incubate with blocking buffer for 30 minutes at room temperature.

4. Wash the cells briefly with PBS and incubate the cells with primary antibodies (1:500 ~ 1:2000) in a blocking buffer for 1 hour at room temperature on a rocking platform.
5. Wash five times with PBS for 2 minutes each on a rocking platform.
6. Incubate the cells with secondary antibodies (for example Alexa-fluor-labeled) (1:1000) in a blocking buffer for 45 minutes on a rocking platform at room temperature in the dark.
7. Decant the secondary antibodies and wash five times with PBS for 2 minutes each in the dark.
8. Observe the fluorescence signal using an inverted confocal microscope or mount the samples with Prolong Gold antifade reagent for long-term storage.