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Immunofluorescence of Cultured Cells

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Abstract

This protocol describes the PFA-fixation and immunofluorescent-labeling of cultured cells.

Subject terms: Cell biology Cell culture Imaging

Keywords: immunofluorescence

Reagents

4% Paraformaldehyde
PBS
PBS + 0.1% TritonX-100
10% serum (from species of secondary)
Primary antibody
Alexa Fluor 488 secondary
Alexa Fluor 568 secondary
DAPI
Mounting media

Equipment

Humidity Chamber

Procedure

1. Wash cells with PBS.
2. Fix in 4% PFA for 15 min at room temperature (RT).
3. Wash cells with PBS (2×5 min). Cells can be stored in PBS at 4C for up to a week.
4. Permeabilize with PBS + 0.1% TritonX-100 for 5 min at RT.
5. Wash with PBS (2×5 min) at RT.
6. Block with 10% serum (from species of secondary) for 1 hr at RT.
7. Drain and incubate with primary antibody diluted in 5% serum overnight at 4C.
8. Wash in PBS (3×5 min) at RT.

9. Detect primary antibody with Alexa Fluor secondary antibody diluted 1:400 in 5% serum at RT for 1 hr in the dark.
10. Wash in PBS (3×5 min) at RT.
11. Add mounting media with DAPI and apply coverslip.
12. Store slides in the dark at 4C.

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Competing financial interests

None.

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Readers' Comments

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