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Immunofluorescence staining and confocal microscopy in cultured cells

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We use this protocol and it's working

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Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

Abstract

This protocol describes immunofluorescence staining and confocal microscopy in lentivirally transduced cultured cells, including fixation, permeabilization, antibody staining, and mounting for fluorescence imaging.

Guidelines

- Antibody dilutions and incubation times may require optimization depending on antibody performance.
- Avoid drying samples during staining and wash steps.
- PLA experiments should follow the manufacturer’s protocol exactly.

Materials

- Lab-Tek II Chamber 8-well slides (Thermo Fisher Scientific, 154534)
- Alexa Fluor 647-conjugated anti-Rabbit IgG (Thermo Fisher Scientific, A-31573)
- Alexa Fluor 488-conjugated anti-Mouse IgG (Thermo Fisher Scientific, A-11001)
- Triton X-100 (Millipore Sigma, X100-500ML)
- 4% paraformaldehyde (Thermo Fisher Scientific, J61899-AK)
- ProLongTM Diamond Antifade Mountant containing DAPI (Thermo Fisher Scientific, P36971)
- glass coverslips (Fisher Scientific, 12541032CA)

Before start

- Perform all staining steps at room temperature unless otherwise indicated.
- Prepare all buffers fresh or thaw on the day of use.
- Protect samples from light after secondary antibody incubation.
- Use appropriate biosafety precautions when handling paraformaldehyde.

Cell seeding

1 Seed lentivirally transduced cells onto Lab-Tek II Chamber 8-well slides (Thermo Fisher Scientific, 154534).

2 Incubate cells for 24 h to reach 40–60% confluency at the time of fixation.

Fixation 20m

3 Rinse cells three times with PBS.

4 Fix cells with 4% paraformaldehyde for 15 min at room temperature. 15m

5 Wash cells three times with PBS. 5m

Permeabilization 10m

6 Permeabilize cells with 0.5% Triton X-100 in PBS for 5 min at room temperature. 5m

7 Wash cells three times with PBS using gentle agitation. 5m

Blocking 1h

8 Block cells with 3% BSA and 0.1% Tween-20 in PBS for 1 h at room temperature. 1h

Primary antibody incubation 2h

9 Dilute primary antibodies in 1% BSA and 0.1% Tween-20 in PBS.

10 Incubate cells with primary antibodies for 2 h at room temperature. 2h

Secondary antibody incubation 5h

11 Dilute secondary antibodies in 1% BSA and 0.1% Tween-20 in PBS:
- Alexa Fluor 647–conjugated anti-Rabbit IgG (1:1000; Thermo Fisher Scientific, A-31573)
- Alexa Fluor 488–conjugated anti-Mouse IgG (1:1000; Thermo Fisher Scientific, A-11001)

12 Incubate cells with secondary antibodies for 1.5 h at room temperature, protected from light. 5h

13 Wash cells three times with PBS.

Mounting

14 Mount cells with ProLong Diamond Antifade Mountant containing DAPI (Thermo Fisher Scientific, P36971).

15 Cover samples with glass coverslips (Fisher Scientific, 12541032CA).

16 Cure mounted slides at room temperature, protected from light, for at least 24 h before sealing with nail polish.

Imaging

17 Acquire images using confocal microscopy under appropriate laser and detector settings.

Proximity ligation assay (PLA) (optional)

18 Perform the proximity ligation assay (PLA) according to the manufacturer's instructions (Sigma Aldrich, DUO92101).