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Immunofluorescent Immunocytochemistry Protocol

1. Solutions and reagents

1.1. Washing buffer:
PBST washing buffer: 1XPBS/0.1% Tween-20 (Dulbecco's Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.)

1.2. 2% Paraformaldehyde (prepare fresh by dissolving paraformaldehyde in 1X PBS by heating at 70 ºC until dissolving. Use cold.)

1.3. 0.1% Triton X-100

1.4. Blocking buffer:
PBS (Dulbecco's Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.) + 10% serum (serum origin depends on the host of the secondary antibody)

1.5. Mounting medium for fluorescence (Vectashield, catalog #H-1000, Vector Laboratories, Inc.).

2. Protocol

2.1. Grow cells in chamber slides or in 6-well tissue plates containing sterile coverslips. Prior to fixation, cells should not reach more than 80% confluency.

2.2. Fixation

2.2.1. Remove medium from chamber slides or 6-well plates. Wash once with PBS-T.
2.2.2. Fix cells with 2% paraformaldehyde for 20 min.
2.2.3. Wash cells twice with 1XPBST.
2.2.4. Permeabilize cells with 0.1% Triton-X100 for 5 min
2.2.5. Wash cells twice with 1XPBST.

2.3. Blocking

2.3.1. Block cells with blocking buffer for 1 hour at room temperature (option - O/N 4 ºC).

2.4. Staining

2.4.1. Dilute primary antibody in the blocking buffer per recommendation on the data sheet.
2.4.2. Apply primary antibody on the cells and incubate overnight at 4 ºC.
2.4.3. Wash cells twice with 1XPBST.
2.4.4. Incubate cells in a dilution of fluorescently-labeled secondary antibody in PBS for 45 min at room temperature in the dark.
2.4.5. Wash cells three times with 1XPBST.
2.4.6. Counterstain cells with DAPI in a concentration of 300 nM in PBS for 5 min in the dark.
2.4.7. Wash cells three times with 1XPBST.
2.4.8. Mount slides with medium for fluorescent staining.
2.4.9. Store slides in the dark.

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