Immunofluorescence Protocol for Adherent Cells on Coverslip

This procedure works well when cells are grown in 6-well tissue culture plates containing sterile coverslips in the appropriate media and concentration of Fetal Bovine Serum (FBS).

Solutions and Reagent

Note: Prepare solutions with Milli-Q or equivalently purified water.

•10X Phosphate Buffered Saline (PBS): 0.58 M sodium phosphate dibasic (Na_2HPO_4), 0.17 M sodium phosphate monobasic (NaH_2PO_4), 0.68 M NaCl. To prepare 1 liter of 10X PBS, use 82.33 g Na_2HPO_4, 23.45 g $NaH_2PO_4.H_2O$ and 40 g NaCl.Adjust pH to 7.4.

•4 % Paraformaldehyde: Prepare day of use either from commercial 16 % stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.

•100 % Methanol

•Tris Buffered Saline (TBS): 50 mM Tris-HCl (pH 7.4), 150 mM NaCl

•0.1 % Sodium Borohydride: Dissolve in PBS on day of use.

•0.2 % Triton X-100: Prepare stock of 20 % Triton in PBS; rotate tube overnight to dissolve. Dilute to 0.2 %

•1 % Bovine Serum Albumin (BSA)

•Blocking Buffer: 10 % horse or goat serum, 1 % BSA, 0.02 % NaN_3, 1X PBS

•ProLong™ Antifade Kit: As directed by Molecular Probes (#P-7481), prepare just before use. Add approximately 1 ml of ProLong mounting medium to one vial of ProLong antifade reagent. Mix gently. Any unused mixture can be stored at –20°C (to slow reaction) for up to one month.

Fixation

1. Prepare 4 % paraformaldehyde and/or cool 100 % methanol to –20°C in a tightly sealed container.

2. Treat cells as desired.

3. Wash cells on coverslips once with cool or room temperature TBS.

4. Aspirate off TBS completely and immediately fix as appropriate for your samples.

Methanol (protein precipitation) Fixation

a. Immerse cells in –20°C 100 % methanol for 10 minutes.

b. Wash slips three times for 5 minutes each with room-temperature TBS. Aspirate off completely and return slips to TBS only. Continue protocol at blocking step.

Paraformaldehyde (cross-linking) Fixation

a. Immerse coverslips in 4 % paraformaldehyde at room temperature for 10 minutes.

b. Wash coverslips once with TBS. Aspirate completely and then permeabilize cells on coverslips with 0.2 % Triton X-100 for 5 minutes at room temperature or alternatively with –20°C methanol for 5 minutes.

c. Wash slips three times for 5 minutes each with TBS at room temperature.

d. Quench cells in fresh 0.1 % sodium borohydride in TBS for 5 minutes. Aspirate off completely and return slips to TBS only. Continue protocol at blocking step.

Blocking

Block all slips with blocking buffer at room temperature for 45–60 minutes. Wash once for 5 minutes with TBS.

Staining

1. Dilute the primary antibody as appropriate in 1 % BSA in TBS. Centrifuging the antibody for 20 minutes at 12,000 x g in a refrigerated microcentrifuge prior to use will remove any aggregated material, thereby reducing background. Apply the diluted antibody to the cells on coverslips and incubate for 1 to 2 hours at room temperature.

   Note: When using any primary or fluorescence-labeled secondary antibody for the first time, titrate out the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

   Note: You may wish to leave one slip for a secondary antibody-only control.

   Note: In some case, You can prolong the incubation time to overnight at 4°C.

2. Wash all slips three times for 5 minutes each with TBS.

3. Incubate all slips with a dilution of the fluorescence-labeled secondary antibody in 1 % BSA in TBS for 30–45 minutes at room temperature in the dark.

4. Wash all slips three times for 5 minutes each with TBS in low lighting.

5. Mount coverslips on slides using the ProLong™ Antifade Kit. Store slides at room temperature in the dark.