Immunofluorescent Staining of Adherent Cell Cultures

Reagents

Poly-D-lysine (Sigma, P6407)
1X PBS
3% Paraformaldehyde in PBS
1X PBS containing 10 mM glycine and 0.2% sodium azide (PBS-GSA)
Triton X-100
Bovine Serum Albumin (BSA)

Procedure

Preparation of cell cultures

1) Soak circular glass coverslips (18 mm) in ethanol and sterilize by flaming.
2) Transfer glass coverslips to 12-well plates and treat with 1 mL/well of 50 μg/mL poly-D-lysine. Incubate at room temperature for 3 hours, remove the poly-D-lysine solution, wash the glass coverslips with sterile water, and air dry.
3) Grow cells on poly-D-lysine-coated coverslips as necessary.

Immunofluorescence staining (all conducted at room temperature unless otherwise noted)

1) Rinse the cells once in PBS (1 mL/well).
2) Fix the cells for 10 minutes using 3% paraformaldehyde in PBS (1 mL/well).
3) Remove the fix solution and quench the reaction by rinsing the cells once with PBS-GSA (1 mL/well). Incubate the cells with fresh PBS-GSA (1 mL/well) for 5 minutes.
4) Permeabilize the cells by treating them with PBS-GSA containing 0.5% Triton X-100 (made fresh) for ***exactly*** 3 minutes.
5) Remove the permeabilization solution and quickly wash the cells with PBS-GSA (1 mL/well). Incubate the cells with fresh PBS-GSA (1 mL/well) for 5 minutes.
6) Dilute the primary antibody in PBS-GSA containing 1% BSA, making only what is needed for the experiment (50 μL/coverslip). Actual dilutions are antibody-dependent, but 1:20 – 1:200 is a typical range. Place 50 μL drops of the primary antibody solution onto a sheet of parafilm. Use forceps to remove each coverslip from the tissue culture plate, remove excess PBS-GSA with a Kimwipe, and place cell side DOWN onto the antibody solution drop. Incubate for 20 minutes.
7) Return the coverslips to the tissue culture plate cell side UP. Rinse quickly with PBS-GSA (1 mL/well). Incubate the cells with fresh PBS-GSA (1 mL/well) for 5 minutes.
8) Dilute the secondary antibody in PBS-GSA containing 1% as with the primary antibody. Typical dilutions for secondary antibodies (Jackson Labs) are:

   FITC-Goat-Anti-Mouse (1:200)
   FITC-Goat-Anti-Rabbit (1:200)
   Texas Red-Goat-Anti-Mouse (1:200)
   Texas Red-Donkey-Anti-Rabbit (1:800)

Place 50 μL drops onto a sheet of parafilm and place coverslips onto them as before. Incubate in the dark for 15 minutes.

9) Return the coverslips to the tissue culture plate cell side UP. Rinse quickly with PBS-GSA (1 mL/well). Incubate the cells with fresh PBS-GSA (1 mL/well) for 10-15 minutes in the dark.
10) To mount each coverslip, place a drop of mounting medium (Vectashield) with or without DAPI onto a microscope slide. Carefully drain and dry off the back of the coverslip with a Kimwipe and place each cell side DOWN onto the mounting medium. Aspirate excess mounting medium from the edges and then use clear nail polish to seal the coverslip edges. Coverslips mounted in this manner can be stored for several months without fading if stored in the dark at 4 °C.