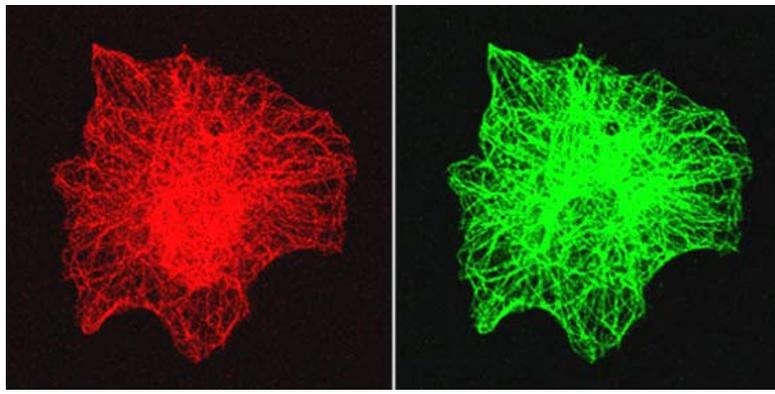


Immunofluorescent Detection of Two Proteins in a Cell

This is a standard procedure for immunofluorescent staining of adherent cells cultured on 12mm glass coverslips in a 24-well tissue culture plate.



Cell Fixation

1. Wash cells on coverslips 3 times with PBS
2. Add 500 μ l 4% paraformaldehyde (in PBS) to each well and incubate for 12 min at room temp
3. Wash 3 times with PBS

Cell Permeabilization

1. Add 500 μ l 1% Triton X-100 (in 0.02% BSA/ PBS)
2. Incubate at room temp for 2 min
3. Wash 3 times with PBS

Blocking

1. Add 500 μ l blocking reagent (20% goat serum in 2% BSA/PBS)
2. Incubate 10 min at room temp
3. Remove block...do NOT wash

Primary Antibody #1 – Mouse Monoclonal

1. Add 250 μ l primary antibody diluted in 2% goat serum in 2% BSA/PBS
2. Incubate at room temperature for 2 hours
3. Wash 3 times with PBS

Secondary Antibody #1 – FITC conjugated goat anti-mouse

1. Add 250 μ l secondary antibody diluted 1:200 in 2% goat serum in 2% BSA/PBS
2. Incubate at room temp for 1 hour
3. Wash 3 times with PBS

Primary Antibody #2 – Rabbit Polyclonal

1. Add 250 μ l primary antibody diluted in 2% goat serum in 2% BSA/PBS
2. Incubate at room temperature for 2 hours
3. Wash 3 times with PBS

Secondary Antibody #2 – Rhodamine conjugated goat anti-rabbit

1. Add 250 μ l secondary antibody (FITC conjugated goat anti-mouse) diluted 1:200
2. Incubate at room temp for 1 hour
3. Wash 3 times with PBS

Nuclear Staining with DAPI

1. Add 1 μ l DAPI stock solution to 10ml PBS
2. Put 250 μ l diluted DAPI solution on cells and incubate at room temp 5 min
3. Wash 3 times with PBS

Mounting Coverslips on a Slide

1. Place coverslip on Kimwipe (cell side up)
2. Put 10 μ l mounting medium on slide and invert drained coverslip into mounting medium and press down gently
3. Store at room temp in the dark

