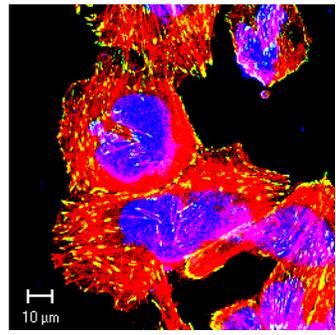
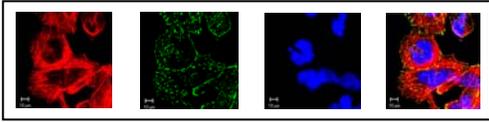


# Focal Adhesions, Stress Fibers and Nuclei

This is a standard procedure for immunofluorescent staining of adherent cells cultured on 12mm glass coverslips in a 24-well tissue culture plate for focal adhesions, stress fibers, and nuclei



## Cell Fixation

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

## Immunofluorescent Staining of Vinculin (focal adhesions)

1. Add 500 $\mu$ l blocking reagent (20% goat serum in 2% BSA/PBS)
2. Incubate 10 min at room temp
3. Remove block...do NOT wash
4. Add 250 $\mu$ l primary antibody (anti-vinculin mouse monoclonal) diluted 1:1000
5. Incubate at room temperature for 1 hour
6. Wash 3 times with PBS
7. Add 250 $\mu$ l secondary antibody (FITC conjugated goat anti-mouse) diluted 1:200
8. Incubate at room temp for 30 min
9. Wash 3 times with PBS

## Direct Staining of F-Actin (stress fibers) with Phalloidin

1. Add 5 $\mu$ l Rhodamine-Phalloidin to 200 $\mu$ l 2% goat serum in 2% BSA/PBS and put on cells in well
2. Incubate at room temp for 30 minutes
3. Wash 3 times with PBS

## Direct Staining of DNA (nucleus)

1. Add 1 $\mu$ l DAPI to 10ml PBS
2. Put 250 $\mu$ l diluted DAPI solution on cells
3. Incubate at room temp 5 min
4. Wash 3 times with PBS

## Mounting Coverslips on a Slide

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