

## Cardiomyocyte immunofluorescent protocols:

Protocols for immunofluorescent staining of cardiomyocytes edited from a protocol kindly provided by:

Dr. Richard Pattern  
Tufts-New England Medical Centre  
Molecular Cardiology Research Centre  
Boston, MA

### Anti-sarcomeric – $\alpha$ actinin and ANP Staining

1. Fix cells as usual in 3.7% paraformaldehyde in PBS.
2. Rinse cells twice with PBS after fixation.
3. Permeabilize and block in 2% FBS / 2% BSA in PBS with 0.1% NP40 for 45 minutes.
4. Treat cells with antibodies for  $\alpha$ -actinin (Abcam alpha sarcomeric actin antibody (alpha Sr-1) ab28052) and ANP diluted in the blocking buffer as above for 1 hour.
5. Wash x3 in PBS for 5 minutes each.
6. Add secondary Ab in the same blocking buffer (for green Ab: CY2 or fluorescein labeled Abs, use 1/200 dilution of secondary Ab and for CY3 or Rhodamine, use 1/1000 dilution for 45 minutes (though these will require some optimization))
7. Wash in PBS X3
8. Add DAPI solution to coverslips for 15 minutes.
9. Wash X2 in PBS.
10. Add mounting solution and coverslip. Image cells within 2 days. The  $\alpha$ -actinin stain is stable for quite some time but the ANP stain tends to bleach out within about 1-2 weeks.

### Immunostaining for ER $\alpha$ or ER $\beta$

1. Fix cells as usual in 3.7% paraformaldehyde in PBS.
2. Rinse cells twice with PBS after fixation.
3. Permeabilize and block in 2% FBS / 2% BSA in PBS with 0.1% NP40 for 45 minutes.
4. Treat cells with antibodies for  $\alpha$ -actinin (1/200 dilution) diluted in the blocking buffer as above for 1 hour.
5. Wash x3 in PBS for 5 minutes each.
6. Then treat cells with anti-ER $\alpha$  (or ER $\beta$ ) antibody at optimized dilution overnight at 4°C.
7. Wash with PBS X3.
8. Treat with secondary antibodies as above (same dilutions) for 45 minutes to an hour.

9. Wash x3 with PBS.
10. Add DAPI solution for 15 minutes.
11. Wash x3 in PBS, then coverslip.