Cardiomyocyte immunofluorescent protocols

Anti-Sarcomeric – α 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 α-actinin (1/200 dilution) and ANP (1/200) diluted in the blocking buffer as above for 1 hour.
5. Wash x3 in PBS for 5 min each.
6. Add secondary Ab in the same blocking buffer (for green Ab: CY2 or fluoroscein 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 3 X.
8. Add DAPI solution to coverslips for 15 min.
9. Wash X2 in PBS.
10. Add coverslip solution and coverslip. Store the slides at 4^{o}C and image cells within 2 days. The α-actinin stain is stable for quite some time but the ANP stain tends to bleach out within about 1-2 weeks.

Immunostaining for ERα or ERb

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 α-actinin (1/200 dilution) diluted in the blocking buffer as above for 1 hr.
5. Wash 3 X in PBS for 5 minutes each.
6. Then treat cells with Anti-ERα (or ERb) antibody at optimized dilution overnight at 4°C.
7. Wash with PBS X3.
8. Treat with secondary antibodies as above (same dilutions) for 45 min to an hour.
9. Wash 3 X with PBS.
10. Add DAPI solution for 15 min.
11. Wash 3 X in PBS, then coverslip.