

Isakson Lab Method 01/05/08

Cremaster Protein Observation

1. expose mouse testicals and immediately immerse in cold 2% PFA
2. continue removing cremaster muscle from mouse and place in 35 mm dish with ice cold 2% PFA
3. place dish on rocker (not rotator) for 10 minutes in cold room
4. bring dish to dissection microscope and open cremasters laterally so that they are opened (i.e., skeletal muscle is exposed) and transfer opened cremasters to new 35 mm dish with 2% PFA for another 10 minutes on rotator
5. transfer cremasters to new 35 mm dish containing PBS and wash 2X 15 minutes on rocker and then 2X 15 minutes on rotator
6. add blocking solution to cremasters on rocker for 2 hours at room temperature
7. move cremaster to new dish and add primary antibody to blocking solution and place on rocker in cold room—parafilm wrap the dish—should be in cold room for 20 hours

8. transfer cremasters to new 35 mm dish containing PBS and wash 2X 15 minutes on rocker and then 2X 15 minutes on rotator
9. add blocking solution to cremasters on rocker for 1 hours at room temperature
10. move cremaster to new dish and add secondary antibody to blocking solution and place on rocker for 2 hours at room temperature [keep covered]
11. transfer cremasters to new 35 mm dish containing PBS and wash 1X 15 minutes on rocker and then 1X 15 minutes on rotator [keep covered]
12. add phalloiden (conjugated to fluorophore of choice) 1:200 to PBS and place on rocker for 60 minutes [keep covered]
13. transfer cremasters to new 35 mm dish containing PBS and wash 1X 15 minutes on rocker and then 1X 15 minutes on rotator [keep covered]
14. pin out cremaster on sylgard and image using PBS on water immersion objective linked to confocal

BLOCKING

2 % BSA

5% fish skin gelatin

5% serum from animal secondary antibody was made in

0.25% Triton X-100

in PBS