Immunocytochemistry for Myosin Light Chain
For Cells in Suspension

Today's Date: 6/2/2006

Protocol

1. From cells in media.. .wash cells twice in freshly-made warm (RT) IX PBS (pH 7.4).

2. Fix cells in freshly made warm 4% formaldehyde for 15-25 minutes. (Dilute 4mL of 16% formaldehyde in 12mL PBS).

3. Wash cells four times in warm IX PBS to remove formaldehyde.

4. Permeablize cells by gently adding ice-chilled 0.1% Triton-X-100 (Dilute 50uL triton X-100 in 50mL PBS) for 5 minutes. Do not disturb or move coverglasses (cells might wash off glass).

5. Wash cells a few times in PBS to remove detergent.

6. Block cells overnight at room temperature in PBS + 2% BSA (Make 2% BSA by dissolving 1 gram of BSA in 50mL warm PBS).

7. Incubate cells for 2 hours in primary antibody (Monoclonal anti-myosin from Sigma 1:200; rabbit ami MLC-P 2032, 1:100) diluted in PBS + 2% BSA. (Make enough for 200uL/coverglass).

8. Wash cells four times in IX PBS for 10 minutes each wash.

9. Incubate cells for 60 minutes in 1:50 secondary AB (Alexa Fluor 488 anti-rabbit and Alexa Fluor 546 mouse) diluted in PBS + 2% BSA. Use 100uL/coverglass.

10. Wash cells four times in PBS for 10 minutes each wash.

11. Mount using ProLong Gold Antifade reagent (Molecular Probes).

12. Image ASAP.