IMMUNOSTAINING & FIXATION OF CELLS - CSB METHOD
(Using Cytoskeleton Stabilization Buffer from Condeelis Laboratory)

Stock Solutions: (make fresh each time)

A. 4% Paraformaldehyde fixative
   20 mls dH2O - hot
   2g paraformaldehyde
   add 3 drops NaOH
   25 mls dH2O
   5 mls 10x PBS

B. 0.1% Igepal
   20 mls CSB solution
   200 uL 1% Igepal

C. 0.1 M Glycine
   100 mls CSB solution
   0.75 g glycine

D. Mounting solution: 0.017g PPD +1.5 ml 7.5% Sodium Bicarbonate NaHCO₃
   + 0.3 ml 0.5M Sodium Carbonate Na₂CO₃
   Add 120 uL above solution + 100 uL 10x PBS + 780 uL 50% glycerol (keep in dark)

E. 250 mls 10x CSB, pH 6.1
   50 mM KCl - 0.93g
   1.37M NaCl - 20g
   40 mM NaHCO₃ - 1.06g
   4 mM KH₂PO₄ - 0.17g
   110 mMNa₂HPO₄ - 3.91g
   20 mM MgCl₂ - 5ml of 1M sol'n

F. 1% BSA
   50 mls 1xPBS/NaN₃
   0.5g BSA

Procedure:

1. Fix cells with 2 mls paraformaldehyde solution - 15 minutes

2. Wash once with 1x CSB solution

3. Permeablize with 0.1% Igepal - 10 minutes

4. Wash once with 0.1M Glycine (check pH of solution first - 6.1)

5. Incubate in 0.1M Glycine for 20 minutes

6. Rinse 3 times in 1x PBS/NaN₃ (quickly)

7. Block with 1% BSA and add Rhodamine/Phalloidin (normally used at 1:1000)- 1ml per dish
   - prepare 1ml aliquots of solution and spin down 5 minutes in microfuge
   - avoid pellet, if any when adding to coverslip
   - incubate 30 minutes at room temperature

8. Add primary antibody + 1ml of BSA per dish. (antibodies normally used at 10mg/ml)
   - prepare 1ml aliquots and spin down 5 minutes, avoiding pellet
   - incubate 1 hour at 37° C

9. Wash 3 times with PBS solution for 5 minutes each time
10. Add secondary antibody + 1ml of BSA per dish. ( 
- prepare 1ml aliquots and spin down 5 minutes, avoiding pellet 
- incubate 30 minutes at 37°C

11. Rinse 1x and wash twice for 5 minutes each time

12. Mount slide: add 10 uL mounting solution to slide, remove solution from dish, carefully blot coverslip and quickly place down on slide. Blot gently, let dry, then seal.