



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

Stock Solutions: (make fresh each time)

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| <p>A. <i>4% Paraformaldehyde fixative</i><br/>20 mls dH<sub>2</sub>O - hot<br/>2g paraformaldehyde<br/>add 3 drops NaOH<br/>25 mls dH<sub>2</sub>O<br/>5 mls 10x PBS</p> <p>B. <i>0.1% Igepal</i><br/>20 mls CSB solution<br/>200 uL 1% Igepal</p> <p>C. <i>0.1 M Glycine</i><br/>100 mls CSB solution<br/>0.75 g glycine</p> <p>D. <i>Mounting solution:</i> 0.017g PPD +1.5 ml 7.5% Sodium Bicarbonate NaHCO<sub>3</sub><br/>+ 0.3 ml 0.5M Sodium Carbonate Na<sub>2</sub>CO<sub>3</sub><br/>Add 120 uL above solution + 100 uL 10x PBS + 780 uL 50% glycerol (keep in dark)</p> | <p>E. <i>250 mls 10x CSB, pH 6.1</i><br/>50 mM KCL- 0.93g<br/>1.37M NaCL - 20g<br/>40 mM NaHCO<sub>3</sub> - 1.06g<br/>4 mM KH<sub>2</sub>PO<sub>4</sub> - 0.17g<br/>110 mM Na<sub>2</sub>HPO<sub>4</sub> - 3.91g<br/>20 mM MgCl<sub>2</sub> - 5ml of 1M sol'n<br/>50 mM PIPES - 3.78g<br/>20mM EGTA-50 mls of 0.1M sol'n<br/>55 mM glucose - 2.48g</p> <p>F. <i>1% BSA</i><br/>50 mls 1xPBS/NaN<sub>3</sub><br/>0.5g BSA</p> |
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### Procedure:

1. Fix cells with 2 mls paraformaldehyde solution - 15 minutes
2. Wash once with 1x CSB solution
3. Permeabilize 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/NaN<sub>3</sub> (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.