

## Fixation and Embedding for Immunocytochemistry

Protocol author: Virginia Weis

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### Supplies

NaPO<sub>4</sub> monobasic and dibasic  
5M NaCl stock solution  
8% glutaraldehyde in ampules  
paraformaldehyde powder  
Methanol  
LR White  
Gelatin capsules

### Solutions

0.2 M Sodium phosphate monobasic  
2.4 g anhydrous Na phos monobasic  
100 ml DH<sub>2</sub>O

0.2 M Sodium phosphate dibasic  
2.84 g anhydrous Na phos dibasic  
100 ml DH<sub>2</sub>O

0.2 M Na phosphate pH 7.2  
approx 28 ml of monobasic  
approx 82 ml of dibasic  
pH the solution to 7.2 with additional mono and di stock

4% paraformaldehyde  
Heat 20 ml of DH<sub>2</sub>O in a small beaker while stirring to just below boiling.  
Add 0.8 g paraformaldehyde powder and stir vigorously. (**Note:** paraformaldehyde is very toxic and should be weighed out with a mask and gloves on. Handle the powder carefully.)  
Add 1-2 drops of 1N NaOH to solution and wait for it to clear. Be patient.  
Check pH with pH paper – if over 7.5 start over.  
Cool solution

Fix: 1% glutaraldehyde, 1% paraformaldehyde, 0.1 M Na phosphate, 0.45 M NaCl pH 7.2  
5 ml 4% paraformaldehyde

10 ml 0.2 M Na phosphate, pH 7.2  
1.8 ml 5M NaCl  
2.5 ml 8% glutaraldehyde  
0.7 ml DH<sub>2</sub>O  
20 ml total  
Prepare this under a hood if possible – fixatives are very toxic

0.1 M Na phosphate, 0.45 M NaCl, pH 7.2  
10 ml 0.2 M Naphos  
1.8 ml 5 M NaCl  
8.2 ml DH<sub>2</sub>O  
20 ml total

### **Procedure**

1. Fix material for 1 hr in fix.
2. Rinse 3 x 10 min in 0.1 M NaPO<sub>4</sub>, 0.45M NaCl pH 7.2
3. Dehydrate 15%, 30%, 50% MeOH for 10 min each
4. Dehydrate in 85%, 95%, 100%, 100% MeOH for 15 min each
5. Place in 1:1 MeOH:LR White overnight on a rotator table in the cold room
6. Place in 100% LR White, for 2 hours at 4 degrees
7. Place at room temp for 4 hours
8. Place fresh LR White into gelatin capsules
9. Place specimens in capsules, seal, and place at 52 degrees for 2 days
10. For immunocytochemistry, have sections placed onto formvar-coated nickel grids

All steps should be performed on a rotating table.