**Perfusion protocol by Martin Wild**

The bird will be perfused first with 0.9% saline and then with a fixative containing 0.5% paraformaldehyde, 1.5% glutaraldehyde, and 1.5% sucrose in 0.1 M phosphate buffer. Mix up to one liter of each.

Saline  
Add 9 grams of NaCl to about 500 ml of ddH20  
Fill to 1 liter  
Filter to remove all large crystals (filter paper must be folded into 1/4 pie, placed in funnel)

Fixative  
Boil about 500 ml of ddH20 in the microwave, which takes about 4 minutes.  
Add ten drops of saturated NaOH  
Place in the hood and add 5 grams of paraformadehyde while stirring for about ten minutes until dissolved  
Cool in ice slurry until it drops below 40 degrees celsius  
Add 250 ml of 0.4 M phosphate buffer  
Add 60 ml of 25% glutaraldehyde  
Bring up to 1 liter with ddH20  
ph to something between 7.4 and 7.5  
filter to remove all crystal conglomerates

During the perfusion, the saline is always on the right and the fix is always on the left.