

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 ddH₂O

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 ddH₂O in the microwave, which takes about 4 minutes.

Add ten drops of saturated NaOH

Place in the hood and add 5 grams of paraformaldehyde 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 ddH₂O

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.