

Fix for Cupric Silver Staining

Perfusion wash:

	g/liter	Final %
NaCl	8	0.8
Dextrose	4	0.4
Sucrose	8	0.8
CaCl	0.23	0.023
Sodium Cacodylate *	0.34	0.034

* Sigma Cat. #C 0250 Cacodylic Acid, sodium salt

150 ml per animals (through the ascending aorta)

Perfusion Fix:

	1000 ml	2000 ml	Final %
Paraformaldehyde	40 g	80 g	4
Sucrose	40 g	80 g	4
Sodium Cacodylate	14.34	28.68	1.4 * (0.067 M)

Filter before using. At least 300 ml per animal.

Following steps are done under fume hood.

- To make the fix, start with approximately 800 ml of water and heat to 60°C on a heater/magnetic stirrer.
- Add the sucrose.
- Create a deep vortex using a magnetic stirrer bar and gradually sprinkle the finely powdered paraformaldehyde onto the vortex surface. The solution will be quite milky.
- Add the Na Cacodylate and the solution will clear considerably but still may be turbid and will require filtration (a qualitative paper is OK) through a large funnel but do so after the solution cools.
- Allow to cool to room temp., bring the total volume to 1000 ml and adjust pH to 7.2-7.4 with concentrated HCl dropwise (ca. 15-20 drops from a Pasteur style pipette).

Dissection:

Wait 12 hours before dissecting the animal. During that period, do not manipulate the carcasses just let them sit at room temperature. After the tissue is removed, put it in the same fixative at 4° C. DO NOT FREEZE!