

## E. M. Primary Fixative.

(2% Gluteraldehyde + 2% Paraformaldehyde in 0.1M Sodium Cacodylate / HCl buffer pH 7.2)

### Protocol.

**All solutions should be prepared immediately before use.**

Three solutions are required in order to prepare the primary fixative.

#### **Solution 1.** 25% Gluteraldehyde.

25% EM Grade Gluteraldehyde. This solution is purchased in 5 ml rubber capped vials and stored in the refrigerator. (seek technical advice).

#### **Solution 2.** 10% Paraformaldehyde.

Prepare this solution by dissolving 10g of Paraformaldehyde powder (*weighed out in the fume hood*) in 80 mls of Millipore grade H<sub>2</sub>O (18M $\Omega$ ). Heat this solution in the fumehood on the hotplate/stirrer to approx. 60°C but not greater than 65°C. Once this temperature has been achieved turn off heat source. Using a plastic pipette, slowly add in 1M NaOH dropwise until the solution clears.

Make up to a final volume of 100 mls. with Millipore grade H<sub>2</sub>O (18M $\Omega$ ) and allow to cool. Filter and store at room temp.in a screwtop bottle which is clearly labelled and dated.

#### **Solution 3.** (0.2M Cacodylate /HCl Buffer pH 7.2)

Prepare a 0.2M Sodium Cacodylate / HCl buffer pH 7.2 solution by dissolving 4.28g (*weighed out in the fume hood*) of Sodium Cacodylate in 80mls of Millipore grade H<sub>2</sub>O (18M $\Omega$ )

Add 8.4 mls. of 0.2 N Hydrochloric Acid and adjust the pH to 7.2 using a standardised pH meter. (*Addition of the acid produces Arsenic gas hence the need for fume extraction*) Bring the final volume up to 100 mls. with dist.H<sub>2</sub>O Store refrigerated in a screwtop bottle which is clearly labelled and dated.

### **To prepare the final working solution.**

Measure out 25 mls of solution 3 (Buffer) and add 10mls of solution 2. (Paraformaldehyde). Using a 5ml syringe and needle, withdraw 4 mls of solution 1 (Gluteraldehyde) and add this in, below the meniscus thus avoiding contact with air, Bring the final volume up to 50 mls. with dist.H<sub>2</sub>O to make the final working solution (mixture of 1, 2 & 3). Check pH the soln. Store refrigerated in a screwtop bottle which is clearly labelled and dated.