

E. M. Secondary Fixative.

(1% Osmium Tetroxide in 0.1M Sodium Cacodylate / HCl buffer pH 7.2)

Protocol.

Initially, two solutions are prepared and an equal volume of both are mixed to yield the final secondary fixative

Solution 1.

Prepare a 2% solution of Osmium Tetroxide by dissolving the contents of 1 ampoule of OsO₄ (0.25g. (250mg)) in 12.5 mls of Millipore dist. H₂O. (2 ampoules in 25 mls is commonly made.)

Dissolution is best done overnight in a brown or opaque wide mouthed bottle in a refrigerator, wrapped in tin foil and clearly labelled as Osmium with a warning of toxicity, and dated. The solution should be kept in the special refrigerator designated for Osmium storage located in the EM Unit.

Note; vapours from this soln. are toxic so all preparatory work should be done under fumehood extraction while wearing vinyl gloves. (Seek technical expertise.)

Solution 2.

Prepare a 0.2 M Sodium Cacodylate / HCl buffer pH 7.2 solution by dissolving 4.28g of Sodium Cacodylate in 100mls of Millipore dist. H₂O.

Add 8.4 mls. of 0.2 M Hydrochloric Acid and adjust the pH to 7.2 using a standardised pH meter.

Final working solution.

As outlined above, to prepare a 1% Osmium Tetroxide in 0.1M Sodium Cacodylate / HCl buffer pH 7.2 solution, simply mix an equal volume of solutions 1 and 2 above to yield the final working solution.

(10 mls of soln. 1 + 10mls. of soln. 2 is commonly used)