

Conventional EM on *Drosophila* Embryos

1. Prepare a 10 ml solution of 25% glutaraldehyde and 2% acrolein contained in 100mM cacodylate buffer, pH 7.4;
2. Add 10 mls of heptane and shake vigorously;
3. Place dechorionated embryos in the heptane phase for 15 min;
4. Remove the excess heptane from the embryos (be careful not to dry) and immerse the embryos in a solution containing 3% formaldehyde (freshly prepared), 2% glutaraldehyde, 0.5% DMSO contained in 100mM cacodylate buffer, pH 7.4; allow to fix an additional 15 min and then begin removing the viteline membrane from the embryos;
5. After de-vitelinizing the embryos ; fix for an additional 2 hrs, rm. temp.:
6. Wash in 0.1M Cac/2.5% sucrose pH 7.4 3X 15' EA;
7. Post-fix with Palade's OsO₄ for two hour on ice, light tight, under hood.
5 ml Palade's 1% OsO₄ : 1 ml Acetate-veronal stock
 + 1.25 ml 4% OsO₄
 + 1 ml 0.1N HCl
 + 1.75 ml ddH₂O

Acetate-veronal stock = 1.15 g NaAcetate Anhydrous
 (J.T. Baker 1-3470)
 + 2.943 g NaBarbituate (Veronal)
 labeled Barbitol--(Sigma B-0500)
 to 100 ml with ddH₂O
8. Rinse 1X with Kellenberger, then incubate for 2 hrs./o.n. at rm. temp. (in the dark).
10 ml Kellenberger = 2 ml Acetate Veronal Stock
 + 2.8 ml 0.1N HCl
 + 5.1 ml ddH₂O
 + 0.05 g Uranyl Acetate
 Check pH with paper before adding UA.
 (Should be ~6)

The following should be done on ice.

9. Cool samples on ice and rinse in cold 50% ethanol for 2.5 min.;

10. Dehydrate with graded series of ethanol (70, 95, 100); move to rm. temp.; and wash 2x/15min.-30 min. in 100% ethanol;

11. Add 100% PO, two changes;

12. Place in 50% PO/50% Epon overnight under vacuum.

13. Embed in 100% Epon. Change two to three times throughout the day --- ~EA hour. (If destroying tissue, don't change Epon.)

14. Put typed or pencil-written label in dummy capsules with wooden stick, at least two capsules per sample. Pour tissue out of tube into mincing dish. Place tissue in flat mold with small amount of Epon (to avoid curling) with a wooden stick and place in 60° oven overnight.