TEM for Drosophila

This protocol was written based on O'Donnell and Bernstein, 1988

Procedures

1. Fixatives do not efficiently permeate the cuticle of adults, and therefore it is necessary to pierce the cuticle or dissect the muscles of interest with a double-edge razor blade before fixation.

2. For IFM and TDT preparations, the head and abdomen are first removed and then thoracic regions not containing the muscle of interest are dissected away. The remaining tissue is placed in fixative containing 3 % paraformaldehyde, 2 % glutaraldehyde, 100 mM sucrose, 100 mM sodium phosphate buffer, pH 7.2, 2 mM EGTA.

3. Samples are treated with the initial fixative overnight at 4°C.

4. Samples are then washed for 45 rain in 100 mM sodium phosphate buffer, pH 7.2.

5. Samples are then treated with a post-fixative (1.0% osmium, 100 mM sodium phosphate, pH 7.2) for 2 h at 4°C.

6. After osmium treatment, samples are washed in distilled water for 45 min at 4°C and then dehydrated in increasing concentrations of ethanol 25%, 50%, 75%, 95%, and 100% twice, acetone 100% twice, 20 min for each at room temperature.

7. Samples are infiltrated with 1:1 acetone-araldite resin at RT for 1 hour.

8. Samples are infiltrated with 1:6 acetone-araldite resin at RT overnight.

9. Samples are infiltrated with fresh araldite resin at RT for 30 minutes, then transfer to 40°C for 1 hour.

10. Samples are infiltrated with fresh araldite resin at 45°C for 1 and ½ hour.

11. Samples are infiltrated with fresh araldite resin at 50°C for 1 and ½ hour.

12. Samples are embedded in fresh araldite resin at 60°C for 24 hours.

13. Sections are cut with a glass or diamond knife (EM Science Corp., Chestnut Hill, MA).