Directions for Processing Biological TEM Samples

NOTE: CARRY OUT ALL WORK INVOLVING FORMALDEHYDE, PROPYLENE OXIDE, OsO_4 AND DMP 30 IN CHEMICAL FUME HOOD. These are hazardous chemicals. Exposure to OsO_4 can cause blindness and death.

Processing Samples (Day 1)

NOTE: All rinse and stain volumes are at least twice the volume of the sample. 1x PBS can be substituted for the 0.1M sodium cacodylate buffer.

1. Fixative is removed and the sample is washed twice with 0.1M sodium cacodylate, at least 10 minutes each wash.

2. Remove 0.1M sodium cacodylate. 1% OsO_4 is added to the sample to completely cover. The tube is sealed and covered (OsO_4 is light sensitive). Stain for one hour.

3. Remove the OsO_4 and rinse with 0.1M sodium cacodylate buffer, 2 times at least 10 minutes each time.

4. Remove sodium cacodylate buffer and rinse with 0.1N acetate buffer, 1 time at least 10 minutes each time.

5. Remove acetate buffer and stain with 0.5% uranyl acetate (UA). Enough UA is added to completely cover the sample. The tube is sealed and covered. Stain for one hour.

6. Remove the uranyl acetate and rinse with 0.1N acetate buffer, 2 times at least 10 minutes each time.

7. After removing the last buffer rinse, wash twice, at least 10 minutes each time, with 30% ethanol.

8. Remove the 30% ethanol and wash twice, at least 10 minutes each time, with 50% ethanol.

9. Remove the 50 % ethanol and wash twice, at least 10 minutes each time, with 70% ethanol.

10. Remove the 70% ethanol and wash twice, at least 10 minutes each time, with 90% ethanol.

11. Remove the 90% ethanol and wash three times, at least 10 minutes each time, with 100% ethanol (200 proof). **If sample is adherent cells or in a plastic container, proceed to Option 2. All other samples follow Option 1.**

NOTE: Propylene oxide dissolves some plastics. Use only glass pipettes or soft plastic transfer pipettes when using PO.

12. **Option 1**
    a. Remove 100% ethanol and add propylene oxide (PO).
    b. Rinse 3 times, at least 10 minutes each, with PO.
    c. Add the resin component, DDSA and NMA together in a plastic beaker (See attached sheet for making resin). Place a stir bar in the plastic beaker and stir until compents are completely mixed together. Add the DMP-30 and continue to stir for at least 10 minutes. Turn off the stirring plate and add a volume of PO equal to the volume in the plastic beaker then stir at low speed. Allow the resin and PO to mix completely. Remove PO from the samples and cover the samples with the 50% resin/50% PO mixture. Allow the samples to sit in hood overnight at room temperature.

13. **Option 2**
    a. Add the resin component, DDSA and NMA together in a plastic beaker (See attached sheet for making resin). Place a stir bar in the plastic beaker and stir. Add the DMP-30 and continue to stir for at least 10 minutes.
    b. Remove the 100% ethanol and add resin. Allow the samples to sit overnight in the hood with the caps off.

Sample Embedding (Day 2)

14. **Option 1**
    a. The following day make up new resin as in Option 2 (day 1), rinse sample in new resin, place sample in molds with new resin. Place molds in oven and bake for at least 48 hours at 50-60°C.

15. **Option 2**
    a. The following day make up new resin as in Option 2 (day 1). Place samples in the 50-60°C oven for at least 10 minutes. Remove resin and replace with new resin. Repeat this step at least once. Replace resin with fresh resin, place molds in oven and bake for at least 48 hours at 50-60°C.

Preparation of Resin

Make the amount of resin required according to the following chart:

Component	Units									
Resin	**grams**	53.3	42.6	40.0	32.0	26.7	21.3	13.3	10.7	5.35
DDSA	**grams**	20.7	16.6	15.6	12.4	10.4	8.3	5.2	4.1	2.05
NMA	**grams**	26.0	20.8	19.5	15.6	13.0	10.4	6.5	5.2	2.60
DMP-30	**mL**	1.4	1.12	1.05	0.84	0.70	0.56	0.35	0.28	0.14
Total Volume	***mL***	***100***	***80***	***75***	***60***	***50***	***40***	***25***	***20***	***10***