

TEM STANDARD PROTOCOL

4% **Paraformaldehyde**

2 g paraformaldehyde
25ml dH₂o
Slowly add ~2 drops of 0.1M NaOH
to clear and filter

0.2 M cacodylate buffer

42.8g Sodium Cacodylate
1000 ml dH₂o
Adjust to pH 7.2

Karnovsky fixative

9.8 ml of 25% glutaraldehyde
18 ml of 0.2 M cacodylate buffer

Osmium post-fixative;

2ml of 2% OsO₄ (osmium tetroxide)
Ted Pella #19152
1 ml of 0.2 M caco buffer

DAY 1

- Fix in **Karnovsky fixative** for 2 hrs at room temperature
- Wash in 0.1M **cacodylate buffer** for 2x15min
- Osmium post fixative** for 2 hrs
- 25% ETOH 2x10min
- 50% ETOH 2x10min
- 70% ETOH 2x10min

HOLD IF YOU NEED TO HERE OVERNIGHT IN THE 4 DEGREE

DAY 2

EPON solution (50 ml)

| | |
|------------------|---------------------|
| EPON 812 – 24 ml | (Ted Pella: #14900) |
| DDSA – 15 ml | (Ted Pella: #13710) |
| NMA – 10.5 ml | (Ted Pella: #19000) |
| DMP 30 - 0.75 ml | (Ted Pella: #13600) |

- 95% ETOH 2x10min
- 100% ETOH 2x10min
- Propylene Oxide 2x10min
- 33% **EPON** in prop. Oxide for 30 min
- 66% **EPON** in prop. Oxide for 1 hr
- 95% **EPON** in prop. Oxide for 1 hr
- 100% **EPON** 1 hr (shaker)
- 100% **EPON** 1 hr (shaker)
- Embed in molds; put in 65C for 4 days

Post-section staining:

5% Uranyl acetate

25 ml dH₂O

25 ml 100% ETOH

2.5g Uranyl acetate

Reynold's Lead Citrate Solution (50 ml):

Bring dH₂O to a boil in the microwave

Cover with Al foil so that it does not get oxygenized

30 ml of dH₂O

1.33 g Lead nitrate

1.76 g Sodium citrate, dihydrate (solution becomes cloudy when sodium citrate is added)

5 ml of 1M NaOH (solution becomes clear when NaOH is added)

add additional 15 ml of distilled water

brief wash 100% ETOH

15 min **5% Uranyl acetate**

brief wash 100% ETOH

brief wash dH₂O

15 min in **Reynold's Lead Citrate Solution**

 Add 5 pellets of NaOH to remove the O₂

brief wash dH₂O

 Add 5 pellets of NaOH to remove the O₂

brief wash dH₂O

Dry with filter paper