

## **General Microwave Processing Protocol (Using 2-ME Buffer to prevent osmium precipitation)**

- 1) Turn on microwave load cooler and check water level in ColdSpot (water recirculator).
- 2) Set Power Lever at 120W.
- 3) Attach vacuum hose to vacuum chamber

*Note: All steps in microwave are done while sample is under vacuum, unless otherwise noted. Polymerization is done without vacuum. No temperature controls are needed, unless otherwise indicated.*

- 4) **Primary fixation:** Approximately 600ul of fixative in microcentrifuge tube.

Processing times and power levels:

- 1) 1 minute, power off
- 2) 40-80 seconds at 120W
- 3) 3 minutes, power off
- 4) 40 seconds at 350W
- 5) Repeat above for difficult-to-infiltrate samples.

- 5) Two 5-minute buffer rinses at room temperature on rocker outside microwave. Followed by three 20-minute 2-ME buffer rinses (0.1M Na Cacodylate, 0.13M Sucrose, 0.01M 2-Mercaptoethanol buffer) for a total of 1hr before osmium fixation.

*Note: 2-ME buffer rinses are to prevent osmium pepper.*

- 6) **OsO<sub>4</sub> Postfixation** (make sure oven is vented to fume hood).

\*Use 2-ME buffer for the osmium dilution.

For samples that are processed in 1.5ml microfuge tubes use 300ul of 2-ME buffer to 100ul of 4% aqueous osmium.

Remove the last buffer rinse from the sample tube and replace with 300ul of 2-ME buffer, then add 100ul of 4% osmium for a final dilution of 1% OsO<sub>4</sub>.

Processing times and power levels:

- 1) 1 minute, power off
- 2) 80 seconds at 120W
- 3) 3 minutes, power off
- 4) 40 seconds at 120W

7) **Immediately** remove samples from the microwave, do three quick rinses (on/off) with 2-ME buffer, then do one 5-minute 2-ME buffer rinse, all at room temp.

*Note: if solution is still turning a dark brown or black do one more rinse if necessary.*

Follow 2-ME rinses with one quick rinse of Milli-Q water (on/off), then three 5-minute Milli-Q water rinses at room temperature on rocker.

8) **Enbloc staining**, 1% aqueous uranyl acetate

*Note: This is an optional step, but can enhance membrane visualization.)*

Processing times and power levels:

- 1) 1 minute, power off
- 2) 40-80 seconds at 120W
- 3) 3 minutes, power off
- 4) 40 seconds at 120W
- 5) Repeat above for difficult-to-infiltrate samples.

9) Three 5-minute Milli-Q water rinses at room temperature on rocker.

*Note: only do this step if the UA step was completed.*

10) **Solvent Dehydration**: 40 seconds each step at 120W

Steps: 1 x 20%, 1 x 50%, 1 x 70%, 1 x 90%, 3 X 100%

Acetone works well and avoids the use of carcinogenic propylene oxide (PO) as a transitional solvent between EtOH and resin. However, if using EtOH, acetone can be substituted for the PO after the 1<sup>st</sup> or 2<sup>nd</sup> 100% EtOH step. Then, continue dehydration with 3 100%-acetone steps before starting infiltration with epoxy resins. If using LR White resin or other water-tolerant resins, begin infiltration after the 2<sup>nd</sup> or 3<sup>rd</sup> 100% EtOH step or even after 70% EtOH depending on your protocol.

**Note:** *If ethanol and PO are used, be careful of PO's volatility. It may be better to avoid vacuum until the 1:1 resin:PO step to avoid violent boiling.*

11) **Infiltration:** 3 minutes each step at 250W

*Note: Number of steps can be increased for difficult samples.*

2:1 solvent: resin  
1:1 solvent: resin  
1:2 solvent: resin  
3 exchanges of pure resin

For each pure resin exchange, after MW step leave sample on rocker for at least 30 minutes at room temperature before embedding for easy-to-infiltrate samples.

*Note: Make sure samples are moving freely in resin and not stuck to bottom of microfuge tube.*

If samples are difficult to infiltrate, try a 4<sup>th</sup> exchange of resin for 60 seconds in the MW and place on rocker overnight before embedding.

For really difficult samples MW samples again in the morning for 3min at 250W and leave on rocker for remainder of day before embedding. Can also MW a couple more times throughout the day before embedding.

## 12) **Polymerize**

Epon/Spurr's, Spurr's and LRW (use gel caps for LRW) in 60C oven for 24 hrs.

Epon and Epon/Araldite resins polymerize for 48hrs.

*Note: LRW & Unicryl resins can be polymerized using UV light.*

Or:

### **Polymerize in microwave with ColdSpot:**

Place plastic dish with specimen holder and capsules on top of water recirculator. Fill container with water to cover capsules (NOT gelatin-caps!). Set power level to at least 650W. Polymerization requires 1 hour and 45 minutes of processing (may vary with different resins, such as resins with an Araldite component). Cover, but do not seal container during microwaving. No temperature control is needed during polymerization when ColdSpot is used. Keep an eye on water level, don't allow to evaporate.

### **Polymerization in microwave without ColdSpot:**

Remove ColdSpot, place plastic dish on floor of microwave and fill with water to cover capsules. Use an 800ml beaker for a water load, which is recirculated by the load cooler.

Polymerization schedule for epoxy resins: 10 minutes at 60C, 10 minutes at 70C, 10 minutes at 80C, and 45 minutes at 100C. Araldite resins may require longer times. Use temperature probe in water with capsules and controller on oven to control temps during polymerization.

Polymerization schedule for LR White or Unicryl: 10 minutes at 60C, 10 minutes at 70C, and 25 minutes at 80C.