



## TEM MICROWAVE PROTOCOL FOR WHOLE EYES

### **Fixative Composition for optic nerve and retina**

2.0% glutaraldehyde, 1.12% paraformaldehyde in 0.13 M Na-cacodylate buffer; with 0.13 mM CaCl<sub>2</sub>, pH 7.40

Process specimens in microwave in vacuum chamber on the recirculating water load (ColdSpot). Power levels and times are abbreviated: MW 120W – “1min off, 80sec on, 3min off & 40sec on at 350W” means “set microwave power at 120 watts and use program for 1 minute power off, 80 seconds power on, 3 minutes power off, then change to 350 watts and process for 40 seconds with power on”.

No temperature control is needed if recirculating water load is used.

2-mercaptoethanol is used to prevent osmium precipitation in tissue.

**Primary fixation:** 120W - 1min off, 80sec on, 3min off, & 40sec on at 350W

### **DAY 1**

- 1). **Buffer Rinses:** 2-ME buffer - 3 rinses x 20 min on rocker.  
(0.01M 2-mercaptoethanol, 0.1M Na Cacodylate buffer, 0.17M sucrose)
- 2). **Secondary fixation:** 1% OsO<sub>4</sub> in 2-ME buffer (example: 300uL buffer:100uL 4% OsO<sub>4</sub>)  
MW 120W, 1 min off, 80 sec on, 3min off, 40sec on.
- 3). **Buffer Wash:** 3 quick (on/off) rinses with 2-ME buffer then 2 x 5min changes on rocker.
- 4). **Milli-Q Water Wash:** 1 quick (on/off) then 3 x 10min Milli-Q water rinses on rocker.
- 5). **Dehydration EtOH:**  
MW 120W, – 40 second on each:  
20%, 50%, 70%, 90%, 95%, 4x100% EtOH
- 6). **Resin Infiltration with LR White:**  
*Caution: Do not use “old” LRW resin as it tends to polymerize during the infiltration steps!*

### **MW 250W, 3min on**

- 2 EtOH:1 LRW - MW then place on rocker for approx 2hr;
- 1 EtOH:1LRW - MW then place on rocker overnight.

## **DAY 2**

### **Continue Resin Infiltration:**

Early morning: 1 EtOH:2 LRW – MW then place on rocker

Between 4 – 5pm 1<sup>st</sup> pure LRW resin exchange – after MW place on rocker overnight

## **DAY 3**

### **Continue Resin Infiltration:**

Early morning: 2<sup>nd</sup> pure LRW resin exchange - after MW place on rocker

Between 4 – 5pm 3<sup>rd</sup> pure LRW resin exchange – after MW place on rocker overnight

## **DAY 4**

### **Continue Resin Infiltration:**

Early morning: 4<sup>th</sup> pure LRW resin exchange - after MW place on rocker

Between 4 – 5pm 5<sup>th</sup> pure LRW resin exchange – after MW place on rocker overnight

### **Continue Resin Infiltration:**

Early morning: 6<sup>th</sup> pure LRW resin exchange - after MW place on rocker

### **7). Embedding and Polymerization:**

4pm<sup>ish</sup> flat embed samples and polymerize for 24hr at 60C.