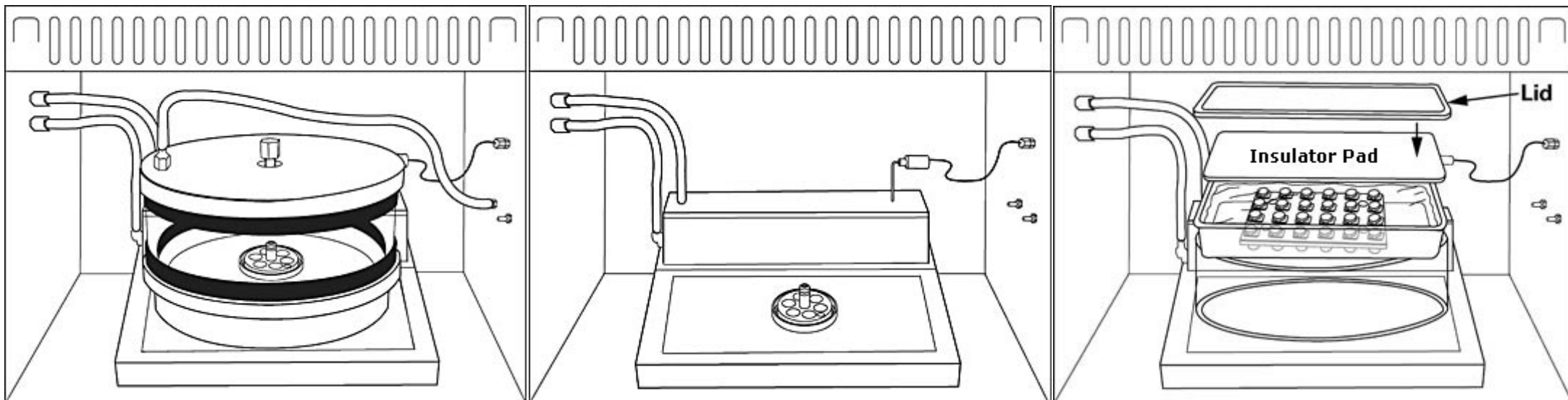


EM Processing Protocol

Microwave Used: PELCO BioWave® Pro - Protocol 1



Microwave Configuration A

Microwave Configuration B

Microwave Configuration C

Step		Wattage	MW Chamber Configuration	Time	Reagent Notes	Process Notes
Fixation Glutaraldehyde	1 step	150W	A	1 min on - 3 min off - 1 min on	Start with fixative at room temperature	Time in Minutes Vacuum 20" Hg
Fixation Karnovsky's or Formaldehyde*	Step 1	150W	A	1 min on - 3 min off - 1 min on	Start with fixative at room temperature	Time In Minutes Wattage change
	Step 2	650W	A	10 sec on - 20 sec off - 10 sec on		Time in Seconds Vacuum 20" Hg
*NOTE: Formaldehyde containing fixatives need a two-step fixation process for fresh tissue						
Fixation Osmium**	Step 1	100W	A	2 min on - 2 min off - 2 min on	Start with fixative at room temperature	Time in Minutes
	Step 2	100W	A	2 min on - 2 min off - 2 min on	Cool fixative if T>27°C before starting Step 2	Time in Minutes

****NOTE:** Osmium penetration into tissue is best at low wattage

Dehydration

50% ETOH	Step 1	<250W	B	40 sec	Start with ETOH or acetone at room temperature
70% ETOH	Step 2	<250W		40 sec	
90% ETOH	Step 3	<250W		40 sec	
100% ETOH	Step 4	<250W		40 sec	
100% ETOH	Step 5	<250W		40 sec	

NOTE: the same steps can be used for acetone in place of ETOH

Resin Infiltration

1:1 acetone:resin	Step 1	>200W	A	3-5 min	Resin viscosity will determine wattage and time	Vacuum 20" Hg
100% resin	Step 2	>200W		3-5 min		
100% resin	Step 3	>200W		3-5 min		

Spurr's & LR White 200W - Epon 250W - Araldite 300W Use longer times based on sample and resin

Resin Polymerization

Epoxy	Step 1	150W	C	30 min	The first 30 minutes of heating should not exceed 60°C for best results	Elevate dish above ColdSpot with vacuum glass ring
Epoxy	Step 2	750W		60 min		
LR White	Step 1	150W		30 min		
LR White	Step 2	750W		15 min		

(1) Use temperature probe to check solution temperature after the microwave step is completed.

The temperature probe will act as a microwave antenna in small fluid volumes creating a sample temperature profile that would not exist otherwise.