**Plant Protocols**

[Basic method - plant tissue](http://stehm.uvic.ca/docs/prep/microwave/protocols.php%22%20%5Cl%20%22basic-planttissue)

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| **Basic method - plant tissue** |

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| Sample type | Plant |
| Subcategory | Regular morphology |
| Specimen(s) | Plant tissue |
| Submitted by | Debra M. Sherman, [Life Science Microscopy Facility](http://www.ag.purdue.edu/facilities/microscopy/pages/default.aspx), Purdue University |
| Instrument used | PELCO 3451 Research Microwave system with PELCO ColdSpot®, vacuum chamber, and variable wattage. |
| Sample size | 1-1.5 mm |
| Sample container | varies |

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| --- | --- | --- | --- | --- |
| **Step** | **Reagent** | **Power** | **Time** | **Vacuum** |
| 1 | Primary fix: 2% PAF + 2.5% glut in cacodylate | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 2 | Wash: 0.1 M cacodylate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 0.1 M cacodylate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 4 | Secondary fix: 2% OsO4 in cacodylate buffer, pH 6.8 | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 5 | Wash: water | 180 W | 40 sec | 5 mm Hg |
| 6 | Wash: water | 180 W | 40 sec | 5 mm Hg |
| 7 | Dehydrate: 30% ETOH | 180 W | 40 sec |  |
| 8 | Dehydrate: 50% ETOH | 180 W | 40 sec |  |
| 9 | Dehydrate: 70% ETOH | 180 W | 40 sec |  |
| 10 | Dehydrate: 90% ETOH | 180 W | 40 sec |  |
| 11 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 12 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 13 | Dehydrate: 100% propylene oxide | 180 W | 40 sec |  |
| 14 | Infiltration: 3 PO : 1 SPURR | 300 W | 3 min on | 5 mm Hg |
| 15 | Infiltration: 1 PO : 1 SPURR | 300 W | 3 min on | 5 mm Hg |
| 16 | Infiltration: 100% SPURR | 300 W | 3 min on | 5 mm Hg |
| 17 | Infiltration: 100% SPURR | 300 W | 3 min on | 5 mm Hg |
| 18 | Infiltration: 100% SPURR | 300 W | 3 min on | 5 mm Hg |
| 19 | Put into beam capsules or flat-bed mold |  |  |  |
| 20 | Polymerization: standard oven at 60 oC |  | 48 hours |  |

##### Detailed reagent prep information

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| Stock buffer | 0.2 M Na-cacodylate buffer (4.28 g / 100 ml H2O, pH 6.8 with HCl  |
| Wash buffer | * 1 part stock buffer
* 1 part dd-H2O
 |
| Primary fix | To make: 20 ml of 1/2 strength Karnovsky's, 2.5% glutaraldehyde + 2% paraformaldehyde (PAF) in 0.1 M cacodylate buffer, pH 6.8 * 6.25 ml of 8% glut
* 3.3 ml of 12% PAF
* 0.25 dd-H2O
* 10 ml of 0.2 M cacodylate
 |
| Secondary fix | To make: 2% OsO4, equal volumes of * 4% OsO4 (stock solution)
* 0.2 M Na-cacodylate buffer
 |

##### Comments

Cacodylate buffer can be replaced by phosphate buffer.

Reduced osmium has also been used for the secondary fixative (1% OsO4 + 1.5% K3Fe(CN)6).

It may be necessary to infiltrate slowly over a 24 (or more) hour period using standard bench-top methods. We often like to do a very slow infiltration by gradually increasing the concentration of the resin until 50% concentration. Then we will use standard step-wise concentrations.

##### References

Karnovsky, M.J. *A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy*, [Journal of Cell Biology](http://jcb.rupress.org) 27:137A (1965)