**Animal Protocols**

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| **Basic method - animal cultured cells** |

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| Sample type | Animal general method |
| Subcategory | Cultured cells |
| Specimen(s) | Varies |
| Submitted by | [Debra M. Sherman](mailto:dsherman@purdue.edu), [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 | Single cell |
| Sample container | varies (culture dishes, etc) |

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| **Step** | **Reagent** | **Power** | **Time** | **Vacuum** |
| 1 | Primary fix: 2% glut in phosphate buffer + salts, pH 7.4 | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 2 | Wash: 0.1 M phosphate buffer, pH 7.4 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 0.1 M phosphate buffer, pH 7.4 | 180 W | 40 sec | 5 mm Hg |
| 4 | Secondary fix: reduced OsO4 in water | 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 | Scrape cells off dishes and embed in 1.5% agarose (Sigma type VII-low temp gelling). Cut pellet into small pieces. |  |  |  |
| 8 | Dehydrate: 30% ETOH | 180 W | 40 sec |  |
| 9 | Dehydrate: 50% ETOH | 180 W | 40 sec |  |
| 10 | Dehydrate: 70% ETOH | 180 W | 40 sec |  |
| 11 | Dehydrate: 90% ETOH | 180 W | 40 sec |  |
| 12 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 13 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 14 | Dehydrate: 100% propylene oxide | 180 W | 40 sec |  |
| 15 | Infiltration: 1 PO : 1 LX-112 | 300 W | 3 min on | 5 mm Hg |
| 16 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 17 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 18 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 19 | Put into beam capsules or flat-bed mold |  |  |  |
| 20 | Polymerization: standard oven |  | 48 hours at 60 oC |  |

##### Detailed reagent prep information

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| Stock buffer | To make: 0.2 M Na-Na2-phosphate buffer, pH 7.4   * 0.2 M Na2HPO4 (approx. 72 ml); adjust to pH 7.4 when adding monobasic * 0.2 M Na2H2PO4 (approx. 28 ml); adjust to pH 7.4 when adding monobasic * Add salts if desired:   + 0.2 M sucrose (6.84 g / 100 ml) or 0.5% NaCl (Adjust amount for desired osmotic regulation)   + 4 mM MgCl2   DO NOT add Ca++ to phosphate buffer |
| Wash buffer | 1 part stock buffer + 1 part dd-H2O |
| Primary fix | To make 20 ml of 2% Glutaraldehyde (glut) in 0.1 M Na-Na2-phosphate buffer, pH 7.4   * 7.5 ml of 8% glut * 2.5 ml dd-H2O * 10 ml of 0.2 M Na-Na2-phosphate buffer, pH 7.4 |
| Secondary fix | To make reduced osmium:   * 1% OsO4 + 1.5% K3Fe(CN)6   [Mix equal volumes of: 2% OsO4 in H2O + 3% K3Fe(CN)6] |

##### Comments

The reagents are just for general information. It all depends on the osmotic need for the specific sample, if it needs to add salt or not for the buffer solution. Also cacodylate buffer can be replaced by phosphate buffer and vise versa.

When using cacodylate buffer, add the following salts to the stock buffer (2X concentrated) if desired:

* 0.2 M sucrose (6.84 g / 100 ml) or 0.5% NaCl (adjust amount for desired osmotic regulation)
* 4 mM MgCl2
* 2 mM CaCl2 [0.02 g / 100 ml or 0.2 ml of 1 M solution (11 g / 100 ml)]

We prefer reduced osmium since we get better membrane contrast. Regular 1% osmium in buffer can be substituted.

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| **Basic method - animal tissue** |

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| Sample type | Animal |
| Subcategory | Basic method for morphology |
| Specimen(s) | Animal tissues |
| Submitted by | [Debra M. Sherman](mailto:dsherman@purdue.edu), [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: 3% glut in phosphate buffer + salts (see comments), pH 7.4 | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 2 | Wash: 0.1 M phosphate buffer, pH 7.4 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 0.1 M phosphate buffer, pH 7.4 | 180 W | 40 sec | 5 mm Hg |
| 4 | Secondary fix: 1% OsO4 in phosphate buffer or reduced osmium (see comments) | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 5 | Wash: 0.1 M phosphate buffer, pH 7.4 | 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: 1 PO : 1 LX-112 | 300 W | 3 min on | 5 mm Hg |
| 15 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 16 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 17 | Infiltration: 100% LX-112 | 300 W | 3 min on | 5 mm Hg |
| 18 | Put into beam capsules or flat-bed mold |  |  |  |
| 19 | Polymerization: standard oven |  | 48 hours at 60 oC |  |

##### Detailed reagent prep information

|  |  |
| --- | --- |
| Stock buffer | To make: 0.2 M Na-Na2-phosphate buffer, pH 7.4   * 0.2 M Na2HPO4 (approx. 72 ml); adjust to pH 7.4 when adding monobasic * 0.2 M Na2H2PO4 (approx. 28 ml); adjust to pH 7.4 when adding monobasic * Add salts if desired:   + 0.2 M sucrose (6.84 g / 100 ml) or 0.5% NaCl (Adjust amount for desired osmotic regulation)   + 4 mM MgCl2   DO NOT add Ca++ to phosphate buffer |
| Wash buffer | 1 part stock buffer + 1 part dd-H2O |
| Primary fix | To make 20 ml of 3% Glutaraldehyde (glut) in 0.1 M Na-Na2-phosphate buffer, pH 7.4   * 7.5 ml of 8% glut * 2.5 ml dd-H2O * 10 ml of 0.2 M Na-Na2-phosphate buffer, pH 7.4 |
| Secondary fix | To make 1% OsO4   * One volume of 4% OsO4 (stock solution) * One volume of H2O * Two volumes of 0.2 M Na-Na2-phosphate buffer, pH 7.4 |

##### Comments

Reduced osmium also has been used often for the secondary fixative (1% OsO4 + 1.5% K3Fe(CN)6), followed by water rinses.

Cacodylate (or other) buffer can replace the phosphate buffer.

When using cacodylate buffer, add following to the stock (2X concentrated) if desired:

* 0.2 M sucrose (6.84 g/ 100 ml) or 0.5% NaCl (osmotic control; adjust % as needed)
* 4 mM MgCl2
* 2 mM CaCl2 [0.02 g / 100 ml or 0.2 ml of 1 M solution (11 g / 100 ml )]

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| **Clinical morphology** |

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| Sample type | Animal, human |
| Subcategory | Clinical morphology |
| Specimen(s) | Kidney, muscle, nerve, tumor, etc |
| Submitted by | [Ronald Austin](mailto:rausti@lsuhsc.edu), Department of Pathology, [LSU Medical Center](http://www.lsuhsc.edu) |
| Instrument used | BioWave™, Ted Pella, Inc. Microwave system with PELCO ColdSpot®, vacuum chamber, and variable wattage. |
| Sample size | 1-2 mm |
| Sample container | varies |

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| **Step** | **Reagent** | **Power** | **Time** |
| 1 | Specimen arrives in Carson-Millonig fixative [1] | 100 W | 2 min |
| 2 | Specimen arrives in Carson-Millonig fixative [1] | 450 W | 30 sec |
| 3 | Wash in buffer choice x3 | 100 W | 1 min each |
| 4 | Secondary fix 1% OsO4 in buffer of choice | 100 W | 5 min |
| 5 | Dehydration: 50% acetone [2] | 250 W | 1 min |
| 6 | Dehydration: 70% acetone [2] | 250 W | 1 min |
| 7 | Dehydration: 95% acetone [2] | 250 W | 1 min |
| 8 | Dehydration: 100% acetone x2 [2] | 250 W | 1 min each |
| 9 | Infiltration: acetone/resin of choice 1:1 | 450 W | 3 min (vacuum) |
| 10 | Infiltration: 100% resin of choice x 2 | 450 W | 3-6 min each (vacuum) [3] |
| 11 | Polymerization in microwave **OR** | 60-90 min [3] | 650 W |
| 12 | Polymerization in conventional oven at 65 o |  | Min 18 hours |

##### Notes

[1] I use a slight variation on the time found in the publication listed below.

[2] Rinse the osmium with a brief wash in 50% acetone and start dehydration with the same percentage. Also, most viscous resins are more soluble in acetone than ethanol; this lab finds the need for propylene oxide unnecessary.

[3] The infiltration and polymerization time depends on the viscosity of the resin. The more viscous the resin, the longer the time in the oven. Use step 11 if you're in a hurry or step 12 if you have a lab schedule to follow.

##### Detailed reagent prep information

0.2 M Sodium cacodylate buffer, pH 7.4, from Polysciences, diluted to a 0.1 M solution.

1% OsO4 one-gram crystals dissolved in same diluted buffer.

Histological grade acetone.

##### References

Giberson, R.T., Austin, R.L.; Charlesworth, J., Adamson, G., and Herrera, G.A. *Microwave and Digital Imaging Technology Reduce Turnaround Times for Diagnostic Electron Microscopy*. Ultrastructural Pathology [27(3):187-196](http://informahealthcare.com/doi/ref/10.1080/01913120309937) (2003)

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| **Vaccinia virus in green monkey cells** |

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| Sample type | Animal |
| Subcategory | Other organinisms |
| Specimen(s) | Faccinia virus in green monkey kidney cells (cultured) |
| Submitted by | [Debra M. Sherman](mailto:dsherman@purdue.edu), [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 | Cell monolayer |
| Sample container | Plastic culture dish |

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| **Step** | **Reagent** | **Power** | **Time** | **Vacuum** |
| 1 | Primary fix: 2% glut cacodylate + salts | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 2 | Wash: 0.1 M cacodylate buffer + salts | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 0.1 M cacodylate buffer + salts | 180 W | 40 sec | 5 mm Hg |
| 4 | Reduced osmium | 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 | Remove cell pellet and enrobe with 2% agarose |  |  |  |
| 8 | Dehydrate: 30% ETOH | 180 W | 40 sec |  |
| 9 | Dehydrate: 50% ETOH | 180 W | 40 sec |  |
| 10 | Dehydrate: 70% ETOH | 180 W | 40 sec |  |
| 11 | Dehydrate: 90% ETOH | 180 W | 40 sec |  |
| 12 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 13 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 14 | Dehydrate: 100% propylene oxide | 180 W | 40 sec |  |
| 15 | Infiltrate: 1 PO : 1 LX-112 + accel | 300 W | 3 min | 5 mm Hg |
| 16 | Infiltrate: 100% LX-112 | 300 W | 3 min | 5 mm Hg |
| 17 | Infiltrate: 100% LX-112 | 300 W | 3 min | 5 mm Hg |
| 18 | Infiltrate: 100% LX-112 | 300 W | 3 min | 5 mm Hg |
| 19 | Put into beam capsules |  |  |  |
| 20 | Polymerize: standard oven at 60 oC |  | 48 hours |  |

##### Detailed reagent prep information

|  |  |
| --- | --- |
| 2X buffer | To make: 100 ml stock solution of 0.2 M Na-cacodylate buffer (4.28 g), pH 7.4   * 4 mM MgCl2 * 6H2O (0.08 g) * 2 mM CaCl2 (0.022 g) * 0.5% NaCl (0.5 g) * pH - 7.4 with HCl |
| Primary fix | To make: 2% gultaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4   * 2.5 ml of 8% glut stock * 5 ml 2x buffer * 2.5 ml H2 |
| Reduced osmium | Use equal volumes of the following:   * 1% OsO4 * 1.5% K3Fe(CN)6 |

##### Comments

Cells looked very good. Membranes were well delineated and mitochondria were satisfactory.

Contrast was adequate with following staining:

* 2 aqueous UA...5 min
* Reynold's Lead Citrate...3 min