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| **Basic method - animal immunocytochemistry** |

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| Sample type | Animal tissues |
| Application | Immunocytochemistry |
| Specimen(s) | Varies |
| 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 + 0.1-0.5% Glut in phosphate buffer (+ salts; see comments) | 180 W300 W | 1 min off, 40 sec on (180 W), 3 min off, 10 sec on (300 W) | 5 mm Hg |
| 2 | Wash: 0.1 M phosphate buffer, pH 7.4 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: water | 180 W | 40 sec | 5 mm Hg |
| 4 | Dehydrate: 30% ETOH | 180 W | 40 sec |  |
| 5 | Dehydrate: 50% ETOH | 180 W | 40 sec |  |
| 6 | Dehydrate: 70% ETOH | 180 W | 40 sec |  |
| 7 | Dehydrate: 90% ETOH | 180 W | 40 sec |  |
| 8 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 9 | Dehydrate: 100% ETOH | 180 W | 40 sec |  |
| 10 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 11 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 12 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 13 | Put into beam capsules or flat-bed mold |  |  |  |
| 14 | Polymerization: vacuum oven filled with nitrogen at 50 °C |  | 24 hours | 5 mm Hg |

##### 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
* If desired add:
	+ 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 | 0.1 M phosphate buffer, pH 7.4 |
| Primary fix | To make 20 ml of 2% paraformaldehyde (PAF) + 0.5% glutaraldehyde (glut) in 0.1 M phosphate buffer, pH 7.4 * 3.33 ml of 12% PAF
* 1.25 ml of 8% glut
* 5.42 dd-H2O
* 10 ml of phsphate stock solution
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##### Comments

Glutaraldehyde % depends on sensitivity of atigen of interest.

Quenching free aldehyde group with 50 mM NH4Cl (0.267 gm / 100 ml of dd water) is an option after the fixation.

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| **Basic method - plant immunocytochemistry** |

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| Sample type | Plant tissues  |
| Application | Immunocytochemistry |
| Specimen(s) | Plant tissue; varies  |
| 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: 3% PAF + 0.1-0.5% Glut in PBS | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 2 | Wash: 20 mM phosphate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 20 mM phosphate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 4 | Wash: 0.1% aqueous toluidine blue in water | 180 W | 40 sec | 5 mm Hg |
| 5 | Wall penetration: 1% Na metaperiodate | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 6 | Quench aldehyde group: 50 mM NH4Cl | 180 W | 1 min off, 40 sec on, 3 min off | 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 | Infiltration: 1 ETOH : 1 LR White | 300 W | 3 min on | 5 mm Hg |
| 14 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 15 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 16 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 17 | Put into beam capsules or flat-bed mold |  |  |  |
| 18 | Polymerization: vacuum oven filled with nitrogen at 50 °C |  | 24 hours | 5 mm Hg |

##### Detailed reagent prep information

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| --- | --- |
| Stock buffer (10x concentration) | To make 0.2 M K-K2-phosphate buffer containing 1.5 M NaCl (8.76 g / 100 ml), pH to 6.8 * Equal amounts of 0.2 M K2HPO4 and 0.2 M KH2PO4 for pH 6.8
* Add 8.76 g of NaCl / 100 ml buffer stock
 |
| Wash buffer | To make 20 mM phosphate buffer (containing 150 mM NaCl), pH 6.8 * 1 part of stock buffer
* 9 parts dd-H2O
 |
| Primary fix | To make 20 ml of modified Van Tuinen, 3% paraformaldehyde (PAF) + 0.5% glutaraldehyde (glut) in 20 mM phosphate buffer (containing 150 mM NaCl), pH 6.8 * 5 ml of 12% PAF
* 1.25 ml of 8% glut
* 11.75 ml of dd-H2O
* 2 ml of phosphate stock solution
 |
| 1% Na metaperiodate | * 1 g / 100 ml of dd-H2O (to perforate cell walls)
 |
| Ammonium chloride | To make 50 mM NH4Cl * 0.267 g / 100 ml dd-H2O (to quench free aldehyde radicals)
 |

##### Comments

Some heavy-walled cells benefit from longer infiltration. This should be done using standard bench-top procedures.

This is an excellent fix for bacteria and other walled organisms.

##### References

Van Tuinen and Reizman, *Immunolocalization of glyceraldehyde-3-phosphate dehydrogenase, hexokinase, and carboxypeptidase Y in yeast cells at the ultrastructural level*. Journal of Histochemistry and Cytochemistry [35(3):327-333](http://www.jhc.org/cgi/content/abstract/35/3/327?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=35&firstpage=327&resourcetype=HWCIT) (1987)

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| **Bacteria immunocytochemistry** |

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| Sample type | Other organisms |
| Subcategory | Immunocytochemistry |
| Specimen(s) | Bacteria, cyanobacteria  |
| 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: 3% PAF + 0.1-0.5% Glut in PBS | 180 W | 1 min off, 40 sec on, 3 min off  | 5 mm Hg |
| 2 | Wash: 20 mM phosphate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 3 | Wash: 20 mM phosphate buffer, pH 6.8 | 180 W | 40 sec | 5 mm Hg |
| 4 | Enrobe using 1.5% low temp gelling agarose (Sigma Type VII); can be done after Step 6 | 180 W | 5 mm Hg |  |
| 5 | Wall penetration: 1% Na metaperiodate | 180 W | 1 min off, 40 sec on, 3 min off | 5 mm Hg |
| 6 | Quench aldehyde group: 50 mM NH4Cl | 180 W | 1 min off, 40 sec on, 3 min off | 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 | Infiltration: 1 ETOH : 1 LR White | 300 W | 3 min on | 5 mm Hg |
| 14 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 15 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 16 | Infiltration: 100% LR White | 300 W | 3 min on | 5 mm Hg |
| 17 | Put into beam capsules or flat-bed mold |  |  |  |
| 18 | Polymerization: vacuum oven at 50 °C filled with nitrogen |  | 24 hours | 5 mm Hg |

##### Detailed reagent prep information

|  |  |
| --- | --- |
| Stock buffer (10x concentration) | To make 0.2 M K-K2-phosphate buffer containing 1.5 M NaCl (8.76 g / 100 ml), pH to 6.8 * Equal amounts of 0.2 M K2HPO4 and 0.2 M KH2PO4 for pH 6.8
* Add 8.76 g of NaCl / 100 ml buffer stock
 |
| Wash buffer | To make 20 mM phosphate buffer (containing 150 mM NaCl), pH 6.8 * 1 part of stock buffer
* 9 parts dd-H2O
 |
| Primary fix | To make 20 ml of modified Van Tuinen, 3% paraformaldehyde (PAF) + 0.5% glutaraldehyde (glut) in 20 mM phosphate buffer (containing 150 mM NaCl), pH 6.8 * 5 ml of 12% PAF
* 1.25 ml of 8% glut
* 11.75 ml of dd-H2O
* 2 ml of phosphate stock solution
 |
| 1% Na metaperiodate | * 1 g / 100 ml of dd-H2O (to perforate cell walls)
 |
| Ammonium chloride | To make 50 mM NH4Cl * 0.267 g / 100 ml dd-H2O (to quench free aldehyde radicals)
 |

##### Comments

Some heavy-walled cells benefit from longer infiltration. This should be done using standard bench-top procedures.

This is an excellent fix for bacteria and other walled organisms.

##### References

Van Tuinen and Reizman, *Immunolocalization of glyceraldehyde-3-phosphate dehydrogenase, hexokinase, and carboxypeptidase Y in yeast cells at the ultrastructural level*. Journal of Histochemistry and Cytochemistry [35(3):327-333](http://www.jhc.org/cgi/content/abstract/35/3/327?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=35&firstpage=327&resourcetype=HWCIT) (1987)

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| **Post-embedded labeling in microwave** |

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| Submitted by | Mark Sanders, Program Director, [Imaging Center](http://www.cbs.umn.edu/ic/), University of Minnesota |
| Instrument used | PTFE spot plate or PELCO® Microwell Staining Mold 170Watt power, Coldspot set to 35°C |

##### Processing

All steps done in microwave at 170W.

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| **Step** | **Reagent** | **Power** | **Time** | **Vacuum** |
| 1 | Rinse sample in phosphate buffer | 170 W | 5 min |  |
| 2 | Decant liquid |  |  |  |
| 3 | Fix cells in 10 volumes of fixative | 170 W | 1 min off, 40 sec at 170W, 3 mins off | 20 in Hg |
| 4 | Wash in phosphate buffer |  | 1 min off, 40 sec at 170W, 3 mins off |  |
| 5 | Wash in 10 mM phosphate buffer + 50 mM NH4Cl  |  | 1 min off, 40 sec at 170W, 3 min off |  |
| 6 | Wash in PBS |  | 1 min off, 40 sec at 170W, 3 min off |  |
| 7 | Dehydrate at 37°C: 25% EtOH | 170 W | 60 sec |  |
| 8 | Dehydrate at 37°C: 50% EtOH | 170 W | 60 sec |  |
| 9 | Dehydrate at 37°C: 75% EtOH | 170 W | 60 sec |  |
| 10 | Dehydrate at 37°C: 95% EtOH | 170 W | 60 sec |  |
| 11 | Dehydrate at 37°C: 2X 100% dry solvent | 170 W | 60 sec |  |
| 12 | Remove water load from vacuum chamber, place samples in dish |  |  |  |
| 13 | Infiltrate 1:1 solvent/LR White resin at 43°C. | 170 W | 2 min |  |
| 14 | Place samples in 100% resin at 43°C | 170 W | 2 min |  |
| 15 | Place samples in 100% resin at 43°C | 170 W | 2 min |  |
| 16 | Place samples in 100% resin at 43°C | 170 W | 2 min |  |
| 17 | Polymerize under water at 47°C |  | 10 min |  |
| 18 | Polymerize under water at 70°C |  | 10 min |  |
| 19 | Polymerize under water at 80°C |  | 25min |  |

##### Labelling

All steps done in PTFE spot plate or PELCO® Microwell Staining Mold 170Watt power, Coldspot set to 35°C.

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| **Step** | **Reagent** | **Power** | **Time** |
| 1 | Wash grids or sections with PBS | 170 W | 40 sec |
| 2 | Wash grids or sections with PBS | 170 W | 40 sec |
| 3 | Apply blocking buffer |  | 2 mins at 170 W, 2 mins off, 2 mins at 170 W |
| 5 | Remove excess serum from the grid by blotting on filter paper |  |  |
| 6 | Apply primary antibody and incubate at 37°C |  | 2 mins at 170 W, 2 mins off, 2 mins at 170 W |
| 7 | Wash with PBS | 170 W | 40 sec |
| 8 | Wash with PBS | 170 W | 40 sec |
| 9 | Wash with PBS | 170 W | 40 sec |
| 10 | Apply secondary antibody and incubate at 37°C |  | 2 mins at 170 W, 2 mins off, 2 mins at 170 W |
| 11 | Wash with PBS | 170 W | 40 sec |
| 12 | Wash with PBS | 170 W | 40 sec |
| 13 | Wash with PBS | 170 W | 40 sec |
| 12 | Rinse well with distilled water, counterstain as usual. |  |  |

##### Detailed reagent prep information

|  |  |
| --- | --- |
| 10 mM phosphate buffer | * 10 ml of 0.1 M phosphate buffer lab stock
* Q.S. to 100 ml with nH2O
* pH to 7.2
 |
| 4% formaldehyde 0.1% glutaraldehyde fixative | * 2.5 ml of 4.0% formaldehyde (16%)
* 0.125 ml of 0.1% glutaraldehyde (8% stock)
* 5.0 ml of 0.1 M phosphate Buffer (0.2 M lab stock)
* Q.S. to 10 ml with nH2O
* pH to 7.2, monitor with pH paper
 |
| 10 mM phosphate buffer + 50 mM NH4Cl | * 10 ml of 0.1 M phosphate buffer
* 0.27 g of NH4Cl (Sigma A-4514, MW 53.5)
* Q.S. to 100 ml with nH2O
 |