

NLGRP Citrus Cryopreservation & Micrografting Media/Solution Formulations

***In vitro* culture media**

Citrus Seed Germination Medium: 2 L

- Murashige & Skoog Basal Salt Mixture (Phytotechnology Labs M524) = 4.33 g
- Sucrose = 50 g
- Myo-inositol (powder) = 0.1 g = 100 mg
- FM Stock (MS ferric EDTA) (200x stock) = 10 mL
- Bring to volume with distilled water
- Agar = 14 g
- pH = 5.8 final
- Dispense 25 mL per 150 X 25 mm glass culture tubes
- Autoclave

Citrus Shoot Tip Recovery Medium: 500 mL

- Lloyd & McCown Woody Plant Basal Mixture (Phytotechnology Labs L154) = 1.15 g
- MS Vitamins (1000x stock) = 0.5 ml
- Sucrose = 25 g
- Bring to volume
- Agar = 3.5 g
- pH = 5.8 final
- Autoclave
- Dispense into 60 X 15mm Petri plates (12 mL/plate)

Citrus Micrograft Recovery Medium: 1 L

- Murashige & Skoog Basal Salt Mixture (Phytotechnology Labs M524) = 4.33 g
- White's vitamin stock (100x stock) = 10 mL
- Sucrose = 75 g
- Bring to volume
- Agar = 7 g
- pH = 5.8
- Dispense 25 mL per 150 X 25mm glass culture tubes w/clear caps
- Autoclave

Stock Solution Recipes for Medium Preparation

FM Stock Solution (MS ferric EDTA) 200x: 500 mL

- Na_2EDTA = 1.865 g (add first, dissolve completely)
- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ = 1.39 g
- Adjust volume to 450 ml
- Boil and allow to cool
- Bring to volume
- Refrigerate at 3-5C in darkness

MS Vitamin Stock (1000x): 30 mL

- Murashige & Skoog Vitamin Powder (1000x) (Phytotechnology Labs M533) = 3.09 g
- Bring to volume
- Dispense into 1 mL aliquots and freeze

White's Vitamin Stock (100x): 250 mL

- Nicotinic acid = 25 mg = 0.025 g
- Pyridoxine HCl = 25 mg = 0.025 g
- Thiamine HCl = 5 mg = 0.005 g
- Myo-inositol = 2500 mg = 2.5 g
- Bring to volume
- Dispense into small vials (10 mL/vial) and freeze

Cryopreservation Solutions

Liquid Preculture Medium, 0.3M Sucrose + ½ MS: 1 L

- Sucrose = 102.69 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22 g
- Bring to volume
- pH = 5.8
- Dispense 25mL per 150 X 25 mm glass culture tubes

Loading Solution, 2M glycerol + 0.4M Sucrose + ½ MS: 1 L

- Glycerol = 184.2 g
- Sucrose = 136.9 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22 g
- Bring to volume
- pH = 5.8
- Dispense 25 mL per 150 X 25 mm glass culture tubes

Plant Vitrification Solution 2 (PVS2): 250 mL

- Glycerol (30% w/v) = 75 g ***weigh this first in flask***
- Ethylene glycol (15% w/v) = 33.8 mL
- DMSO (dimethyl sulfoxide) (15% w/v) = 34.1 mL
- Sucrose (0.4 M) = 34.25 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 0.55 g
- Bring to volume
- pH = 5.8
- Filter sterilize using 0.45 micron syringe filter or Stericup filter units
- Dispense into sterile glass or plastic tubes, seal and refrigerate

Unloading Solution, 1.2 M Sucrose + ½ MS: 1 L

- Sucrose = 410.76 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 2.22 g
- Bring to volume
- pH = 5.8 final
- Dispense 25 mL per 150 X 25 mm glass culture tubes

Shoot Tip Dip/Rinse Solution, 3% sucrose + MS solution: 1 L

- Sucrose = 30 g
- Murashige & Skoog Basal Medium with Vitamins (Phytotechnology Labs M519) = 4.43 g
- Bring to volume
- pH = 5.8
- Dispense 25 mL per 150 X 25 mm glass culture tubes

NLGRP PVS2 Cryoprotectant Preparation, 250 mL

- 1) Weigh out **75 g of glycerol** in a 250 mL Erlenmeyer flask
- 2) Place a stir bar into flask and put flask onto a stir plate
- 3) While stirring, add **33.8 mL of ethylene glycol** using a graduated pipette
- 4) Add **34.1 mL of DMSO** using a graduated pipette
- 5) Add **34.25 g of sucrose**
- 6) Add distilled water to bring the volume in flask to ~225 mL
- 7) Add **0.55 g of MS Salts + Vitamins powder** (M519, Phytotechnology Labs or use MS stock solutions)
- 8) **Bring to final volume of 250 mL** using a graduated cylinder
- 9) **Adjust pH up or down** to 5.8 using 0.5 M potassium hydroxide or hydrochloric acid solutions, respectively
- 10) **Filter sterilize** using .45 μ m Stericup filter unit or syringe filter
- 11) Transfer into sterile plastic or glass containers, seal and refrigerate

PVS2 Cryoprotectant composition for 250mL

- Glycerol (30% w/v) = 75 g ***weigh this first in flask***
- Ethylene glycol (15% w/v) = 33.8mL
- DMSO (dimethyl sulfoxide) (15% w/v) = 34.1mL
- Sucrose (0.4 M) = 34.25g
- Phytotechnology Labs M519 (MS salts + vitamins) = 0.55g
- pH=5.8