

NLGRP Prunus Cryopreservation Media/Solution Formulations

In vitro culture media

Prunus Shoot Maintenance Medium (1L)

- [Murashige & Skoog Basal Medium with Vitamins](#) (Phytotechnology Labs M519) = 4.43 g
- Sucrose= 30 g
- BAP (0.1 mg/mL stock)= 2.5 mL
- Kinetin (0.1 mg/mL stock)= 5 mL
- Bring to volume
- Agar= 7 g
- pH= 5.7 final

Prunus Preculture Medium (250 mL)

- [Murashige & Skoog Basal Medium with Vitamins](#) (Phytotechnology Labs M519) = 0.55 g
- Sucrose (0.3 M)= 25.7 g
- Proline (0.4 M)= 11.51 g
- Glutathione, reduced form (Phytotechnology Labs G3399) (1 mM)= 19.22 mg
- Salicylic acid (0.1 mM) (1mg/mL stock)= 3.45 mL
- Bring to volume
- Agar= 2 g
- pH= 5.7 final

Prunus Recovery Medium #1 (-NH₄) (500 mL)

- [Murashige & Skoog Modified Basal Salt Mixture](#) (Phytotechnology Labs M571) (-NH₄)= 0.67 g
- [Murashige & Skoog Micronutrient Salt Base](#) (Phytotechnology Labs M554)= 0.025g
- MS vitamins (1000x stock)= 0.5 mL
- Sucrose= 15 g
- BAP (0.1 mg/mL stock)= 0.5 mL
- Kinetin (0.1 mg/mL stock)= 0.5 mL
- GA3 (0.1 mg/mL stock = 0.5 mL
- Bring to volume
- Agar= 4 g
- pH= 5.7 final

Prunus Recovery Medium #2 (+NH₄) (500 mL)

- [Murashige & Skoog Macronutrient Salt Base](#) (Phytotechnology Labs M502)= 1.1 g
- [Murashige & Skoog Micronutrient Salt Base](#) (Phytotechnology Labs M554)= 0.05 g
- MS vitamins (1000X)= 0.5 mL
- Sucrose= 15 g
- BAP (0.1 mg/mL stock)= 1.25 mL
- Kinetin (0.1 mg/mL stock)= 1.25 mL
- GA3 (0.1 mg/mL stock)= 0.5 mL
- Bring to volume
- Agar= 4g
- pH= 5.7 final

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

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

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 250 mL

- 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