**Media recipes for *Allium***

**Gamborg B5 basal medium+NAA+2iP** (solid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| Gamborg B5 basal medium[1] | 3.21 g (prepackaged as G398[2]) |
| 2iP [6-(y,y-dimethylallylamino)purine] | 0.05 mg |
| NAA (1-naphthaleneacetic acid) | 0.01 mg |
| Sucrose | 30.0 g |

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with $ddH_20$
- ✓ Adjust pH to 5.7
- ✓ Add:

| | |
|---|---|
| Agar (Sigma®[3] A2700*) | 6.5 g |

- ✓ Heat and stir until boiling
- ✓ For Magenta®[4] GA7* culture vessels dispense prior to autoclaving (60 ml medium/GA7 vessel). For Petri dishes, dispense medium in a laminar flow hood after autoclaving.
- ✓ Autoclave

**Recipes for *Allium* vitrification Method 1: PVS2**

**2 M glycerol+0.6 M sucrose+1/2 MS loading medium** (liquid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| MS basal salt mixture[5] | 2.165 g (prepackaged as M524[2]) |
| Glycerol (w/v) | 184.2 g |
| Reagent grade sucrose | 205.4 g |
| MS-G[6] | 2.5 ml |
| MS-vitamins[6] | 10.0 ml |

- ✓ Heat and stir until sucrose is completely dissolved
- ✓ Bring to final volume (1000 ml) with $ddH_20$
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

**MS[5]-G stock solution** (liquid) – 250 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:
- ✓

| | |
|---|---|
| Thiamine HCl | 0.02 g |
| Myo-inositol | 5.0 g |

- ✓ Bring to final volume (250 ml) and stir until well blended
- ✓ Aliquot into vials ( 2.5 ml/vial)
- ✓ Store in freezer until needed

**MS[5]-vitamin stock solution** (liquid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| Glycine (free base) | 0.2 g |
| Myo-inositol | 10.0 g |
| Nicotinic acid (free base) | 0.05 g |
| Pyridoxine HCl | 0.05 g |
| Thiamine HCl | 0.01 g |

- ✓ Stir until ingredients are well blended
- ✓ Bring to final volume (1000 ml) with ddH20
- ✓ Dispense into desired vessel and store at 5°C

**PVS2** (liquid) – 100 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| Glycerol | 30.0 g (w/v) |
| Ethylene glycol | 13.5 ml |
| Reagent grade sucrose | 13.7 g |
| DMSO (dimethylsulfoxide) | 13.6 ml |

- ✓ Stir until sucrose is completely dissolved
- ✓ Bring to final volume (100 ml) with $ddH_20$
- ✓ Adjust pH to 5.7

- ✓ Filter sterilize

**1.2 M sucrose+1/2 MS medium** (liquid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| MS basal medium w/vitamins[5] | 4.43 g (prepackaged as M519[2]) |
| Reagent grade sucrose | 410.76 g |

- ✓ Heat and stir until sucrose is completely dissolved
- ✓ Bring to final volume (1000 ml) with $ddH_20$
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

**Recipes for *Allium* vitrification Method 2: PVS3**

**2 M glycerol+0.4 M sucrose+1/2 MS loading medium** (liquid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| MS basal medium w/vitamins[5] | 2.21 g (prepackaged as M519[2]) |
| Glycerol (w/v) | 184.2 g |
| Reagent grade sucrose | 136.92 g |

- ✓ Heat and stir until sucrose is completely dissolved
- ✓ Bring to final volume (1000 ml) with $ddH_20$
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

**PVS3** (liquid) – 100 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| Glycerol | 50.0 g (w/v) |
| Reagent grade sucrose | 50.0 g |

- ✓ Heat and stir until sucrose is completely dissolved
- ✓ Bring to final volume (100 ml) with $ddH_20$
- ✓ Dispense into desired vessels
- ✓ Filter sterilize or autoclave

**1.2 M sucrose+1/2 MS medium** (liquid) – 1000 ml

- ✓ To a small volume of double distilled water ($ddH_20$) add:

| | |
|---|---|
| MS basal medium w/vitamins[5] | 4.43 g (prepackaged as M519[2]) |
| Reagent grade sucrose | 410.76 g |

- ✓ Heat and stir until sucrose is completely dissolved
- ✓ Bring to final volume (1000 ml) with $ddH_20$
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

[1]Gamborg et al., 1968
[2]Phytotechnology Laboratories, Shawnee Mission, KS*
[3]Sigma-Aldrich, St. Louis, MO*
[4]Magenta Corp. Chicago, IL*
[5]Murashige & Skoog, 1962
[6]Recipe follows

* Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.