Media recipes for *Rubus*

Blackberry multiplication medium (solid) – 1000ml

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

MS basal medium w/vitamins[1]	4.43g (prepackaged as M519[2])
Sucrose	30.0g
BA (6-benzylaminopurine)	1.0mg
IBA (indole-3-butyric acid)	0.1mg
GA_3 (gibberellic acid)	0.1mg

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

Gellan gum (Phytagel™[3*])	1.45g
Agar (Bacto™[4*])	3.5g

- ✓ Heat and stir until boiling and clear
- ✓ Dispense into Magenta®[5] GA7* culture vessels (75ml/vessel)
- ✓ Autoclave

Blackberry pretreatment medium with 5% DMSO (solid) – 1000ml

Dimethylsulfoxide (DMSO) is heat labile and must be filter sterilized, then added to autoclaved medium, cooled to about 50-55 °C. When preparing this medium allow room in the vessel for the 50 ml/1000 ml DMSO to be added after autoclaving.

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

MS basal medium w/vitamins[1]	4.43g (prepackaged as M519[2])
Sucrose	30.0g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to volume with ddH_20 leaving room for DMSO to be added after autoclaving
- ✓ Adjust pH to 5.7
- ✓ Add:

Agar (Bacto™[4*])	8.0g

- ✓ Stir until blended (the agar in this recipe will dissolve in the autoclave)
- ✓ Dispense into desired vessels

- ✓ Autoclave
- ✓ Allow media to cool in a 55°C water bath
- ✓ Add:

  Filter sterilized DMSO: 50ml (5%)

- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

3.0% sucrose MS (liquid)

To a small volume of double distilled water add:

- ✓ Murashige & Skoog 4.44 g/L
  [M519 Phytotechnology Labs* Murashige & Shoog Basal medium w/ vitamins; contains macro- and micronutrients, and vitamins as described by Murashige & Soog (1962); Plant Tissue Culture Tested]
- ✓ Sucrose (3% use table sugar) 30g/L
- ✓ Stir until dry ingredients are completely dissolved.
- ✓ Bring up to volume.
- ✓ pH 5.7
- ✓ Dispense into desired vessels.
- ✓ Autoclave

PGD (slow cooling cryoprotectant) - 50ml

Add:

- ✓ 3.0% sucrose liquid MS (no hormones): 30.0 ml
- ✓ DMSO: 4.9 ml
- ✓ D-glucose (dextrose): 5.0 g add slowly while stirring
- ✓ Polyethylene glycol 10,000 (PGD): 5.0 g add slowly while stirring
- ✓ Add more liquid MS, keeping the total volume under 50ml.
- ✓ Stir until dissolved.
- ✓ Remove stir bar and bring up to volume with 3.0 % sucrose liquid MS
- ✓ Filter sterilize.

Blackberry recovery medium (solid) – 1000ml

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

MS basal medium w/vitamins[1]	4.43 g (prepackaged as M519[2])
Sucrose	30.0 g
BA (6-benzylaminopurine)	1.0m g
GA_3 (gibberellic acid)	0.1m g

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

Agar (Bacto™[4*])	3.5 g

- ✓ Heat and stir until boiling and clear
- ✓ Dispense into Magenta®[5] GA7 *culture vessels (75ml/vessel)
- ✓ Autoclave
- ✓ Allow media to cool slightly and then dispense medium into sterile Petri dishes (60X15mm). Alternatively, the medium can be stored in the GA7 culture vessels, then reheated and dispensed on an as needed basis.
- ✓ [1]Murashige & Skoog, 1962
- ✓ [2]Phytotechnology Laboratories, Shawnee Mission, KS*
- ✓ [3]Sigma-Aldrich, St. Louis, MO*
- ✓ [4]Becton, Dickenson and Company, Sparks, MD*
- ✓ [5]Magenta Corp., Chicago, IL*

*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.