Media Recipes for *Mentha*

MS growth medium (solid) – 1000 ml

- ✓ 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

- ✓ 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*])	1.3 g
Agar (Bacto™[4])	3.0 g

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

Media Recipes for *Mentha* cryopreservation: Method 1

MS+0.06 M sucrose medium (liquid) - 1000 ml

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

MS basal medium w/vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	20.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

MS+0.3 M sucrose medium (liquid) – 1000 ml

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

MS basal medium w/vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	102.69 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

MS+2 M glycerol+0.4 M sucrose medium (liquid) – 1000 ml

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

MS basal medium with vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	136.92 g
Glycerol	184.18 g (w/v)

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

PVS2 (liquid) – 250 ml

- ✓ Combine:

Glycerol	75.0 g (w/v)
Ethylene glycol	33.8 ml
DMSO (dimethylsulfoxide)	34.1 ml
Reagent grade sucrose	34.25 g
MS basal medium w/vitamins[1]	1.1 g (prepackaged as M519[2])

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (250 ml) with double distilled water (ddH_20)
- ✓ Adjust pH to 5.8
- ✓ Filter sterilize

MS+1.2 M sucrose medium (liquid) – 1000 ml

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

MS basal medium w/vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	411.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

MS+BA+IBA recovery medium (solid) – 1000 ml

- ✓ 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)	0.5 mg
IBA (indole-3-butyric acid)	0.1 mg

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

Agar (Bacto™[4*])	7.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

Media Recipes for *Mentha* cryopreservation: Method 2

MS+5% DMSO (solid) – 1000 ml
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 vessel space 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 with vitamins[1]	4.43 g (prepackaged as M519[2])

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:

Gellan gum (Phytagel™[3*])	1.3 g
Agar (Bacto™[4*])	3.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ Wait for medium to slightly cool and add 50 ml of filter sterilized DMSO in a laminar flow hood. Mix thoroughly.
- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

MS+2 M glycerol+0.4 M sucrose medium (liquid) – 1000 ml

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

MS basal medium with vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	136.92 g
Glycerol	184.18 g (w/v)

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

PVS2 (liquid) – 250 ml

- ✓ Combine:

Glycerol	75.0 g (w/v)
Ethylene glycol	33.8 ml
DMSO (dimethylsulfoxide)	34.1 ml
Reagent grade sucrose	34.25 g
MS basal medium w/vitamins[1]	1.1 g (prepackaged as M519[2])

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (250 ml) with double distilled water (ddH_20)
- ✓ Adjust pH to 5.8
- ✓ Filter sterilize

MS+1.2 M sucrose medium (liquid) – 1000 ml

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

MS basal medium w/vitamins[1]	4.43 g (prepackaged as M519[2])
Reagent grade sucrose	411.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

MS+BA+IBA recovery medium (solid) – 1000 ml

- ✓ 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)	0.5 mg
IBA (indole-3-butyric acid)	0.1 mg

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

Agar (Bacto™[4*])	7.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

Media Recipes for *Mentha* cryopreservation: Method 3

Ca-free MS growth medium (solid) – 1000 ml

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

Potassium nitrate (KNO_3)	1.9 g
Ammonium nitrate ($NH_4\ KNO_3$)	1.65 g
Magnesium sulfate ($MgSO_4$)	0.1807 g
Potassium phosphate, *monobasic* (KH_2PO_4)	0.17 g
Iron stock[6]	10.0 ml
MS micronutrients[1,6]	10.0 ml
MS vitamins[1,6]	10.0 ml
Sucrose	30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.8
- ✓ Add:

Gellan gum (Phytagel™[3*])	1.25 g
Agar (Bacto™[4*])	3.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

3% alginic acid+0.2 M sucrose medium (liquid) – 1000 ml

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

Reagent grade sucrose	68.46 g

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

Low viscosity (250 cps) Na-alginic acid (Sigma®[3] A0682*)	30.0 g

To prevent clumping, add the alginic acid slowly to rapidly stirring medium. A homogenizer with a propeller-type stirring blade works well for this step.

- ✓ Continue stirring until well blended and alginic acid is completely dissolved (~ 20 minutes)
- ✓ Dispense into desired vessels
- ✓ Autoclave

Iron stock solution (100x) (liquid) – 500 ml

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

  NaEDTA, disodium salt, dihydrate ($NA_2EDTA*2H_20$) 1.86 g

- ✓ Stir until NaEDTA is completely dissolved
- ✓ In a separate vessel containing a small volume of ddH_20 add:

  Ferric sulfate ($FeSO_4*7H_2O$) 1.39 g

- ✓ Heat and stir until the ferric sulfate is completely dissolved. Allow solution to cool completely
- ✓ Combine the NaEDTA solution with the ferric sulfate solution
- ✓ Bring to volume (500 ml) and stir until the solution turns yellow
- ✓ Dispense into an amber vessel to prevent photodegredation. Store at 2-4°C

MS[1] micronutrient stock solution (100x) (liquid) – 500 ml

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

Boric acid (H_3BO_3)	0.31 g
Cobalt chloride ($CoCl_2 * 6H_20$)	0.00125 g
Cupric sulfate ($CuSO_4*5H_20$)	0.00125 g
Zinc sulfate ($ZnSO_4* 7H_20$)	0.43 g
Molybdic acid, sodium salt, dihydrate ($NaMo0_4* 2H_20$)	0.0125 g
Manganese sulfate ($MnSO_4*H_20$)	0.845 g
Potassium iodide (KI)	0.0415 g

- ✓ Heat and stir until boiling and dry ingredients have completely dissolved
- ✓ Bring to final volume (500 ml) with ddH_20
- ✓ Dispense into desired vessel and store at 2-4 °C or aliquot and store in freezer

MS[1] vitamin stock solution (100x) (liquid) – 500 ml

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

Glycine (free base)	0.1 g
Myo-inositol	5.0 g
Nicotinic acid (free base)	0.025 g
Pyridoxine HCl	0.025 g
Thiamine HCl	0.005 g

- ✓ Stir until ingredients are well blended
- ✓ Bring to final volume (500 ml) with ddH20
- ✓ Dispense into desired vessel and store at 2-4 °C or aliquot and store in freezer

0.2 M sucrose+0.1 M calcium chloride medium (liquid) – 1000 ml

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

Reagent grade sucrose	68.46 g
Calcium chloride, dihydrate ($CaCl_2*2H_20$)	14.7 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

0.2 M sucrose medium (liquid) – 1000 ml

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

Reagent grade sucrose	68.46 g

- ✓ 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

0.75 M sucrose medium (liquid) – 1000 ml

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

Reagent grade sucrose	256.73 g

- ✓ 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

0.5 M sucrose medium (liquid) – 1000 ml

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

Reagent grade sucrose	171.16 g

- ✓ 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 medium (liquid) – 1000 ml

- ✓ 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

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.7
- ✓ Dispense into desired vessels
- ✓ Autoclave

MS+BA+IBA recovery medium (solid) – 1000 ml

- ✓ 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)	0.5 mg
IBA (indole-3-butyric acid)	0.1 mg

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

Agar (Bacto™[4])	7.0 g

- ✓ Mix and heat until boiling
- ✓ Autoclave
- ✓ In laminar flow hood, dispense liquid medium into sterile Petri dishes (60X15 mm)

- ✓ [1]Murashige & Skoog, 1962
- ✓ [2]Phytotechnology Laboratories, Shawnee Mission, KS*
- ✓ [3]Sigma-Aldrich, St. Louis, MO*
- ✓ [4]Becton Dickinson & Co., Franklin Lakes, NJ*
- ✓ [5]Magenta Corp. Chicago, IL
- ✓ [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.