

Media Recipes for *Humulus*

***Humulus* growth medium I (HGI) (solid) - 1000 ml**

- ✓ To a small volume of double distilled water (ddH₂O) add:

Potassium nitrate (KNO ₃)	1.9 g
Ammonium nitrate (NH ₄ KNO ₃)	1.65 g
Magnesium sulfate (MgSO ₄)	0.1807g
Calcium chloride, <i>dihydrate</i> (CaCl ₂)	0.332 g
Potassium phosphate, <i>monobasic</i> (KH ₂ PO ₄)	0.17 g
Sequestrene 138™ ^{1*} , iron chelate (EDDHA)	0.1 g
Iron stock ²	20.0 ml
MS micronutrients ^{2,3}	10.0 ml
MS vitamins ^{2,3}	10.0 ml
Glucose	20.0 g
BA (6-benzylaminopurine)	0.1 mg

- ✓ Stir until well blended
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Adjust pH to 5.7
- ✓ Add:

Gellan gum (Phytigel™ ^{4*})	1.75 g
Agar (Sigma® ⁴ A7002*)	3.5 g

- ✓ Heat and stir until boiling
- ✓ Dispense into stacked Magenta®⁵ GA7* culture vessels (40 ml/vessel)
- ✓ Autoclave

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

- ✓ To a small volume of double distilled water (ddH₂O) add:

NaEDTA, disodium salt, dihydrate (NA₂EDTA*2H₂O) 1.86 g

- ✓ Stir until NaEDTA is completely dissolved
- ✓ In a separate vessel containing a small volume of ddH₂O add:

Ferric sulfate (FeSO₄*7H₂O) 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 photodegradation. Store at 2-4°C

MS³ micronutrient stock solution (100x) (liquid) – 500 ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

Boric acid (H ₃ BO ₃)	0.31 g
Cobalt chloride (CoCl ₂ * 6H ₂ O)	0.00125 g
Cupric sulfate (CuSO ₄ *5H ₂ O)	0.00125 g
Zinc sulfate (ZnSO ₄ * 7H ₂ O)	0.43 g
Molybdic acid, sodium salt, <i>dihydrate</i> (NaMoO ₄ * 2H ₂ O)	0.0125 g
Manganese sulfate (MnSO ₄ *H ₂ O)	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 ddH₂O
- ✓ Dispense into desired vessel and store at 2-4 °C or aliquot and store in freezer

MS³ vitamin stock solution (100x) (liquid) – 500 ml

- ✓ To a small volume of double distilled water (ddH₂O) 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 ddH₂O
- ✓ Dispense into desired vessel and store at 2-4 °C or aliquot and store in freezer
- ✓

Humulus growth medium II (HGII) (solid) - 1000 ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

Potassium nitrate (KNO ₃)	1.9 g
Ammonium nitrate (NH ₄ KNO ₃)	1.65 g
Magnesium sulfate (MgSO ₄)	0.1807 g
Calcium chloride, <i>dihydrate</i> (CaCl ₂)	0.332 g
Potassium phosphate, <i>monobasic</i> (KH ₂ PO ₄)	0.17 g

Iron stock ²	20.0 ml
MS micronutrients ^{2,3}	10.0 ml
MS vitamins ^{2,3}	10.0 ml
Glucose	20.0 g
BA (6-benzylaminopurine)	0.1 mg

- ✓ Stir until well blended
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Adjust pH to 5.7
- ✓ Add:

Gellan gum (Phytigel™ ^{4*})	1.75 g
Agar (Sigma® ⁴ A7002*)	3.5 g

- ✓ Heat and stir until boiling
- ✓ Dispense into stacked Magenta®⁵ GA7* culture vessels (40 ml/vessel)
- ✓ Autoclave

Ca-free MS+3% (w/v) Na-alginate medium (liquid) – 100 ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

Potassium nitrate (KNO ₃)	0.19 g
Ammonium nitrate (NH ₄ KNO ₃)	0.165 g
Magnesium sulfate (MgSO ₄)	0.018 g
Potassium phosphate, <i>monobasic</i> (KH ₂ PO ₄) ¹	0.017 g
Iron stock ¹	1.0 ml
MS micronutrients ^{1,3}	1.0 ml
MS vitamins ^{1,3}	1.0 ml
Sucrose	17.1 g
BA (6-benzylaminopurine)	0.1 mg
GA ₃ (gibberellic acid)	0.01 mg

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (100 ml)
- ✓ Adjust pH to 5.0
- ✓ Add:

Alginate sodium salt, 2% viscosity (Sigma⁴ A2158*) 3.0 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 min.)
- ✓ Dispense into desired vessels
- ✓ Autoclave

100 mM calcium chloride+MS encapsulation medium (liquid) – 1000 ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

MS basal medium w/vitamins ³	4.43 g (pre-packaged as M519 ⁶)
Calcium chloride (CaCl ₂), dehydrate	14.7 g
Sucrose	30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Adjust pH to 5.0
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

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

- ✓ To a small volume of double distilled water (ddH₂O) add:

MS basal medium w/vitamins ³	4.43 g (prepackaged as M519 ⁵)
Sucrose	256.72 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Adjust pH to 5.0
- ✓ Mix and heat until boiling
- ✓ Dispense into desired vessels
- ✓ Autoclave

Ca-free MS medium (liquid) - 1000ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

Potassium nitrate (KNO ₃)	1.9 g
Ammonium nitrate (NH ₄ KNO ₃)	1.65 g
Magnesium sulfate (MgSO ₄)	0.1807 g
Calcium chloride, dihydrate (CaCl ₂)	0.332 g
Potassium phosphate, <i>monobasic</i> (KH ₂ PO ₄)	0.17 g
Iron stock ²	20.0 ml
MS micronutrients ^{2,3}	10.0 ml
MS vitamins ^{2,3}	10.0 ml

- ✓ Adjust pH to 5.0
- ✓ Stir until well blended
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Dispense into desired vessels
- ✓ Autoclave

Humulus recovery medium (solid) – 1000 ml

- ✓ To a small volume of double distilled water (ddH₂O) add:

Potassium nitrate (KNO ₃)	1.9 g
Ammonium nitrate (NH ₄ KNO ₃)	1.65 g
Magnesium sulfate (MgSO ₄)	0.1807 g
Calcium chloride, dihydrate (CaCl ₂)	0.332 g
Potassium phosphate, <i>monobasic</i> (KH ₂ PO ₄)	0.17 g
Iron stock ¹	20.0 ml
MS micronutrients ^{1,3}	10.0 ml
MS vitamins ^{1,3}	10.0 ml
Glucose	20.0 g
GA ₃ (gibberellic acid)	0.05 mg
BA (6-benzylaminopurine)	0.5 mg

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH₂O
- ✓ Adjust pH to 5.0
- ✓ Add:

Gellan gum (PhytageI™ ^{4*})	1.75 g
Agar (Sigma® ⁴ A7002*)	3.5 g

- ✓ Heat and stir until well blended
- ✓ Autoclave
- ✓ In laminar flow hood, dispense slightly cooled liquid into Petri dishes
- ✓ ¹Becker Underwood Inc., Ames, IA*
- ✓ ²Recipe follows
- ✓ ³Murashige & Skoog, 1962
- ✓ ⁴Sigma-Aldrich, St. Louis, MO*
- ✓ ⁵Magenta Corp., Chicago, IL*
- ✓ ⁶Phytotechnology Laboratories, Shawnee Mission, KS*

*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.