Media Recipes for *Humulus*

Humulus growth medium I (HGI) (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 ($MgSO_4$)	0.1807g
calcium chloride, *dihydrate* ($CaCl_2$)	0.332 g
potassium phosphate, *monobasic* (KH_2PO_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 ddH_20
- ✓ Adjust pH to 5.7
- ✓ Add:

gellan gum (Phytagel™[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

[1] *Becker Underwood Inc., Ames, IA*
[2] *Recipe follows*
[3] *Murashige & Skoog, 1962*
[4] *Sigma-Aldrich, St. Louis, MO*
[5] *Magenta Corp., Chicago, IL.*

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^3 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[3] 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

Humulus growth medium II (HGII) (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
calcium chloride, *dihydrate* ($CaCl_2$)	0.332 g
potassium phosphate, *monobasic* (KH_2PO_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 ddH_20
- ✓ Adjust pH to 5.7
- ✓ Add:

gellan gum (Phytagel™[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 (ddH_20) 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, *monobasic* (KH_2PO_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:

alginic acid sodium salt, 2% viscosity (Sigma[4] 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 minutes)

✓ Dispense into desired vessels

✓ Autoclave

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

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

MS basal medium w/vitamins[3]	4.43 g (pre-packaged as M519[6])
calcium chloride ($CaCl_2$), *dihydrate*	14.7 g
sucrose	30.0 g

- ✓ Stir until dry ingredients are completely dissolved
- ✓ Bring to final volume (1000 ml) with ddH_20
- ✓ Adjust pH to 5.0
- ✓ Heat and stir until well blended
- ✓ Dispense into desired vessels
- ✓ Autoclave

[6] *Phytotechnology Laboratories, Shawnee Mission, KS*

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

- ✓ To a small volume of double distilled water (ddH_20) 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 ddH_20
- ✓ 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 (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
calcium chloride, *dihydrate* ($CaCl_2$)	0.332 g
potassium phosphate, *monobasic* (KH_2PO_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 ddH_20
- ✓ Dispense into desired vessels
- ✓ Autoclave

Humulus recovery 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
calcium chloride, *dihydrate* ($CaCl_2$)	0.332 g
potassium phosphate, *monobasic* (KH_2PO_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 ddH_20
- ✓ Adjust pH to 5.0
- ✓ Add:

gellan gum (Phytagel™[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