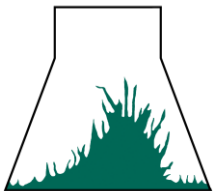


HOSTA MULTIPLICATION KIT

Product No. H411



PhytoTechnology Laboratories®

Mailing Address: P.O. Box 12205, Shawnee Mission, KS 66282-2205

Phone: 1-888-749-8682 (1-913-341-5343 *Outside the USA & Canada*)

Fax: 1-888-449-8682 (1-913-341-5442 *Outside the USA & Canada*)

Visit our Web Site at <http://www.phytotechlab.com>

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KIT COMPONENTS

Product No.	Product Description	Quantity
	Box	1
	Instruction Manual	1
C215 – 10 ea	Culture Containers	1
F951 – 1 ea	Forceps, 8”	1
S963 – 1 ea	Scalpel Handle, No.3	1
S971	Scalpel Blades, No. 11	2
P334 – 1 roll	pH Strips, 4.5 – 7.5	1
D940 – 20 ea	Petri Dishes	1
V886 – 15 mL	Vinegar	1
S803 – 25 g	Sodium Bicarbonate (Baking Soda)	1
P068	Pipette, Plastic Transfer	2
H435 – 1L	Hosta Initiation/Multiplication Medium	3
H436 – 1L	Hosta Multiplication Medium	3
H437 – 1L	Hosta Rooting Medium	3
H3959 – 1L	Hosta Initiation/Multiplication Medium II	3
B130 – 100 mL	Benzyladenine (BA) Solution (1mg/mL)	1
Optional at an additional charge	Hosta Culture (NOT included in kits sent outside the continental USA)	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Beakers/containers: three 250-ml
2. Media preparation container
3. Tissue culture grade water (e.g., distilled/ deionized)
4. 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. P720)
5. 1000 mL of sterile distilled water (Product No. W783)
6. 70% Isopropyl alcohol
7. Nylon mesh
8. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
9. Hosta plant(s) – Actively growing with young (2-8 cm tall) shoots

INTRODUCTION

The purpose of this kit is to demonstrate clonal micropropagation of Hostas from the multiplication of pre-formed meristems (shoot tips) or from the formation of adventitious shoots from flower stalk (scape) tissue. Hostas are commercially micropropagated which allows for the rapid multiplication and production of new plants. This technique produces many more plants than propagation by crown division. Micropropagation of Hostas can be done by 2 methods: 1) Shoot tip (or meristem) culture where shoots are generated from preformed meristems (analogous to terminal and lateral buds), and by 2) Adventitious shoot formation from young flower stalks (scapes). This latter method is not advised for variegated plants as adventitious shoots will typically have only one color, due to the way adventitious shoots form.

MICROPROPAGATION STAGES

Stage 1 — Cultures initiated from shoot tip tissue (explant) and growth begins. Medium used: Hosta Initiation/ Multiplication Medium (Product No. H435)

Stage II — Multiplication phase where the explant multiplies forming numerous shoots. Medium used: Hosta Multiplication Medium (Product No. H436) or Hosta Initiation/Multiplication Medium II (Product No. H3959)

Stage III — Typically the rooting phase where individual shoots are stimulated to form roots. Medium used: Hosta Rooting Medium (Product No. H437)

STOCK PLANT TREATMENT

A bottle of Benzyladenine (BA) solution is included in this kit. BA is a cytokinin that stimulates the growth of lateral buds. It is routinely used in 8 micropropagation media for many plants to stimulate shoot multiplication. BA can also be applied to plants to overcome apical dominance (where the terminal bud or shoot tip produces an auxin that suppresses the growth of lateral buds down the stem). This BA solution can be applied to Hosta plants to induce multiple shoots to grow from the crown.

MEDIA PREPARATION

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. If possible, the entire contents of each package should be used immediately after opening. Media stored at 2 to 6 °C and tightly sealed should last 2-3 years. Preparing the medium in a concentrated form is not recommended as some salts in the medium may precipitate. The basic steps for preparing the culture medium are listed below:

1. Measure out approximately 90% of the desired final volume of tissue culture grade water, e.g. 900 mL for a final volume of 1000 mL. Select a container twice the size of the final volume.
2. While stirring the water, add the powdered medium and stir until completely dissolved.
3. Rinse the container that the medium was packaged in with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in Step 2.
4. Add agar while stirring; it will not dissolve but should disperse into a uniform suspension.
5. The media provided in this kit are complete and typically do not require other supplements; however, an additional supplement such as BA solution (Product No. B130) can be added to the medium if desired.
6. Add additional tissue culture grade water to bring the medium to the final volume.
7. While stirring, determine the pH using the pH Strips (Product No. P334). If necessary, adjust the medium to the desired pH using the baking soda to raise the pH or vinegar to lower the pH. A pH of 5.6 to 5.8 is typically recommended for most plants, including hosta. Alternatively, the pH can be adjusted by using dilute potassium hydroxide or sodium hydroxide solution to raise the pH and dilute hydrochloric (muriatic) acid to lower the pH of the medium.
8. While stirring, heat the solution to nearly boiling to melt the agar in the medium.
9. Dispense the medium into the culture vessels before or after autoclaving as indicated below:
 - a. The Petri dishes (Product No. D940) included in this kit are sterile and cannot be autoclaved. They will melt if heated in an autoclave (or pressure cooker). Medium to be dispensed in Petri dishes must be sterilized and partially cooled before pouring it in the dishes.
 - b. The culture vessels (Product No. C215) are autoclavable. Media should be dispensed in these vessels prior to sterilization in an autoclave or pressure cooker. The lids of these culture vessels C215 should not be tightly sealed during sterilization to allow for proper steam and pressure penetration.
10. Sterilize the medium in a validated autoclave or pressure cooker at 1 kg/cm², 121 °C (15 psi, 250 ° F), for the time period described under “Sterilization of Media” below.
11. Allow medium to cool prior to use.

STERILIZATION OF MEDIA

Plant tissue culture media are generally sterilized by autoclaving at 121 °C and 1.05 kg/cm² (15 psi). This high temperature not only kills bacteria and fungi, but also their heat-resistant spores. Media can be sterilized in either an autoclave or pressure cooker with similar results. The time required for sterilization depends upon the volume of medium in the vessel. The minimum times required for sterilization of different media volumes are listed below. It is advisable to dispense medium in small aliquots whenever possible as many media components are broken down by prolonged exposure to heat.

MEDIA STERILIZATION TIMES

Volume of Medium per Vessel (mL)	Minimum Autoclaving Time (min.)
25	15-20
50	25
100	28
250	31
1000	40
2000	48
4000	63

Please Note: Minimum Autoclaving Time includes the time required for the liquid volume to reach the sterilizing temperature (121 °C) and 15 minutes at 121 °C (Burger, 1988). Times may vary due to differences in autoclaves. Validation with your autoclave or pressure cooker is recommended.

CULTURE PROCEDURES

Establishing Hosta Cultures from Isolated Shoot Tips

1. Wipe down all surfaces of the transfer hood or work area with 70% isopropyl alcohol. Allow the hood to run for 15 min before beginning transfer operations. Place all necessary materials under the hood, e.g. Petri dishes, fresh culture medium. Place scalpels and forceps in a 250-ml beaker containing about 150 mL of 70% isopropyl alcohol.
2. Remove the crown(s) from the soil when the plant(s) start to break dormancy and new shoots are 2-8 cm tall (before the first leaves unfold).
3. Gently wash off all soil from the crown.
4. To isolate the meristem (growing point) cut off the shoot and a small portion of the basal stem (crown) tissue.
5. Gently remove the layers of petioles and leaves until only a small point, the shoot tip, about 1 – 1.5 cm long remains. Cut the basal stem away until about 0.5 cm thickness remains. Be certain that any discolored areas are removed as these could be signs of bacterial or fungal contamination. The smaller the amount of tissue remains, the less chance there is for endogenous (internal) contaminants.
6. Repeat this process until the desired number of shoot tips has been isolated.
7. Place the shoot tips in a 250-mL beaker containing tap water and cover with nylon mesh. Place it under running tap water so that the water gently agitates the shoot tips. Adding a small amount of detergent may be helpful in the cleaning process. Allow the shoot tips to rinse in this manner for 5-10 min. Once the shoot tips have been washed thoroughly, pour off all water, remove the nylon mesh, and transfer the beaker of tissue to the laminar flow hood.
8. Pour enough 70% ethanol over the shoot tips to cover them, swirl for 3-4 min, and decant the ethanol. Next pour the bleach solution over the shoot tips and sterilize for 10-15 min before decanting off the solution. Rinse the shoot tips three times in sterile distilled water with each rinse lasting approximately 1 min.
9. Place the culture vessels containing the media in the hood/work area.
10. All tools which now contact the shoot tips should be sterilized in alcohol and then flamed to remove any alcohol.
11. Transfer one shoot tip to a sterile Petri dish. Cut a thin slice from the basal stem tissue to

remove that which was in contact with the bleach. Place the freshly cut surface of the shoot tip into contact with the medium in a culture vessel. The surface of the stem can be poked just under the surface of the medium but be sure NOT to bury the shoot tip in the medium. One to several shoot tips can be placed in each culture vessel. Once all cultures have been completed, place them in low light (e.g., fluorescent light) at 25° C.

12. Once shoots have developed they can be subcultured (individually transferred) onto fresh medium for continued multiplication on Hosta Multiplication Medium (Product No. H436), Hosta Initiation/Multiplication Medium II (Product No. H3959) or transferred to Hosta Rooting Medium (Product No. H437).
13. Be patient as many months of subculturing may be required to achieve rapid multiplication rates.

Establishing Hosta Cultures from Flower Scapes

1. Remove young emerging flower stalks from the center of the plant.
2. Wash and surface sterilize in 10% bleach as previously outlined.
3. Under sterile conditions in a laminar flow hood, transfer each flower scape to a sterile Petri dish and section it into approx. 3 cm segments.
4. Place the segments on the surface of medium in culture vessels. These may be placed flat (longitudinally) or vertically. Some references promote placing them vertically upside down for greatest success. You can experiment with different orientations, but be sure to label the culture vessels which way the flower scape sections are placed on the medium.

Multiplication of Established Cultures

1. An established stage II (multiplying) culture is included with the kit. This can be used to demonstrate the concept of micropropagation without having to establish cultures from meristems or flower scapes.
2. All work should be performed under sterile conditions in a laminar flow hood as previously outlined for culture establishment. Wipe the outside of the stage II culture container with 70% isopropyl alcohol and place it in the hood along with fresh media, sterile Petri dishes, and a sterile forceps and scalpel.
3. Remove the Hosta shoot mass from the medium and place it on a sterile Petri dish.
4. Using the forceps and scalpel, cut or break apart the base of the mass into individual shoots.
5. Place these shoots onto fresh multiplication medium; 2-4 shoots can be placed in each container of medium.
6. Replace the lid of the container(s) and put the culture(s) under fluorescent light as previously indicated for new cultures.
7. Subculture (break shoots apart and transfer to fresh medium) as desired. This is typically done at 30 – 60 day intervals to maintain actively growing cultures.

APPROXIMATE SCHEDULE

Event	Timing
Isolation of fresh explants	Day 0
First appearance of explant-derived shoots	Day 60 - 90
Transfer to soil	Day 90 – 120 (When plantlets are large enough to handle)

LITERATURE CITED

Burger, D.W. 1988. Guidelines for autoclaving liquid media used in plant tissue culture. HortScience 23:1066-1068.

MEDIA FORMULATIONS

All components express in mg/L	Hosta Initiation/ Multiplication Medium	Hosta Multiplication Medium	Hosta Initiation/ Multiplication Medium II	Hosta Rooting Medium
COMPONENT	H435	H436	H3959	H437
Ammonium Nitrate	1650	1650	1650	1650
Boric Acid	6.2	6.2	6.2	6.2
Calcium Chloride, Anhydrous	332.2	333	332.2	332.2
Cobalt Chloride·6H ₂ O	0.025	0.025	0.025	0.025
Cupric Sulfate·5H ₂ O	0.025	0.025	0.025	0.025
Na ₂ EDTA	37.26	37.26	37.26	37.26
Ferrous Sulfate·7H ₂ O	27.8	27.8	27.8	27.8
Magnesium Sulfate	180.7	180.7	180.7	180.7
Manganese Sulfate·H ₂ O	16.9	16.9	16.9	16.9
Molybdc Acid (Sodium Salt)·2H ₂ O	0.25	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	0.83	0.83
Potassium Nitrate	1900	1900	1900	1900
Potassium Phosphate, Monobasic	300	300	300	300
Sodium Phosphate Monobasic	170	170	170	170
Zinc Sulfate·7H ₂ O	8.6	8.6	8.6	8.6
Adenine Hemisulfate	160	160	160	-
Agar	8000	8000	8000	8000
6-Benzylaminopurine (BA)	2	0.1	2	0.1
Glycine (Free Base)	2	2	2	-
myo-Inositol	100	100	100	100
a-Naphthaleneacetic Acid	0.5	0.5	0.02	0.5
Sucrose	30000	30000	30000	30000
Thiamine·HCl	0.4	0.4	0.4	0.4
Grams of powder to prepare 1 liter	43.40	43.39	43.40	43.23
pH ± 0.5 at RT	4.3	4.3	4.3	4.3

STOCK SOLUTION AND MEDIA PREPARATION LOG

Product Number: _____ Medium: _____

Lot Number: _____ Prepared By/ Date: _____

Volume to Prepare: _____ Autoclave Sterilization Time: _____

pH Desired: _____ Actual Final pH: _____

Instructions: Complete the table with all components of the stock solution or medium to be prepared, including the product number, lot number, and grams/batch. As each component is weighed record the actual weight on the sheet. Check off each component after it is added to the solution/medium.

Component	Product Number	Lot Number	Grams/ Batch	Actual Weight	Added <input checked="" type="checkbox"/>
					<input type="checkbox"/>
					<input type="checkbox"/>
					<input type="checkbox"/>
					<input type="checkbox"/>
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Instructions/ Comments:

Species/Tissue Cultured: _____

NOTES

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