ORCHID STEM PROPAGATION KIT

Product No. O755

***Phyto*Technology Laboratories®**
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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
P748 – 1L	Orchid Maintenance/Replate Medium	3
B141 – 1L	BM-1 Terrestrial Orchid Medium	3
B142 – 1L	BM-2 Terrestrial Orchid Medium	3
O753 – 1L	Orchid Multiplication Medium	3

MATERIALS REQUIRED BUT NOT PROVIDED

1. Beakers/containers: three 250-ml
2. Media preparation container
3. Tissue culture grade water (Product No. W783 is sterile deionized water)
4. 5-10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. P720)
5. 70% Isopropyl alcohol
6. Bunsen or alcohol burner (Product No. B966 or B876, respectively)

INTRODUCTION

*Phyto*Technology Laboratories' Orchid Stem Propagation Kit is designed to allow the user to propagate selected orchid species and hybrids by lateral buds on floral spikes. Included are four media (Product No. P748, B141, B142, and O753), which enables the user to evaluate different media with the goal of determining the best ones for their species or hyrbid of interest. The four media provided in this kit are ready-to-use formulations.

Because of the variety of orchids cultured, media are frequently supplemented with different additives (e.g., fruit extracts, organic compounds, and inorganic salts) to optimize it for specific species. These additives can alter the pH of the medium. Typically, orchid media is adjusted to a pH of 5.0 to 5.6. This kit contains pH indicator strips to evaluate the pH of the media, and baking soda (sodium bicarbonate) and vinegar to raise or lower the pH, respectively, to the desired value.

PROTOCOL

1. Remove any flowers that may remain on the flower stalk. Use clean, healthy, vigorous flower stalks with buds in their nodes. Stalks on which only a few flowers have bloomed are best. Avoid old flower stalks.
2. Wash the flower stalk under running tap water for 5 minutes.
3. Prepare a 10% commercial bleach solution and add 2-3 drops of Tween-20 (Product No. P720) to this solution.
4. Section the flower stalk into smaller section by using a clean razor blade or scalpel cutting between the nodes. Cut the flower stalk into 12 – 20 mm (1/2 – 3/4") sections leaving about 6 mm (1/4") below the node and the remainder above the node.
5. Place the nodal section in the commercial bleach solution (Step 3) for 15 minutes. Swirl the solution every 2-3 minutes.
6. After surface sterilizing, discard the bleach solution and carefully remove the bract from around the node using aspectic methods.
7. Prepare a 5% commercial bleach solution and add 2-3 drops of Tween-20 (Product No. P720) to this solution.
8. Once all of the bracts have been removed, surface sterilize the nodes in the 5% commercial bleach solution prepared in Step 7. Keep in this solution for 10 minutes, swirling the solution every 2-3 minutes.
9. Remove all of the bleach solution and rinse the nodes with sterile distilled water. Rinse by pouring the water over the nodes, swirling, then pour off the water. Repeat this step three times.
10. Under sterile conditions, remove approximately 3 mm (1/8") from each end of the nodal sections using a clean, sterile scalpel or razor. All tools should be dipped frequently in alcohol and flamed with a alcohol lamp (Product No. B910) or Bunsen burner (Product No. B966), or heated in a glass bead sterilizer to maintain sterility.
11. Typically, nodal sections are transferred to the culture vessels containing Orchid Multiplication Medium (O753). The other media (P748, B141, B142) may also be tried. Insert the longer portion of the nodal section into the medium at a slight angle to just below the bud. This should result in the emerging shoot pointing upwards.
12. Most shoots will generally appear within one month and they are ready for replating after about 60 days.
13. Many nodes exudates phenolic compounds into the media which may turn the media dark brown to black. This phenolic exudation will kill your nodes if you do not replate them to fresh media. The use of media containing charcoal will reduce the frequency of replating as

the charcoal absorbs and binds the phenolic compounds.

14. Replate onto Orchid Maintenance Medium (P748) or BM-1 (B141) or BM-2 (B142) media and allow the plantlets to continue to develop and root. Roots will begin to appear after 2 or 3 leaves have been produced.

REPLATING SHOOTS FOR MULTIPLICATION OR CONTINUED GROWTH

1. Approximately 60 days after culturing the bud, it will be necessary to transfer the shoots to fresh media for continued growth or multiplication.
2. Prepare orchid multiplication or maintenance/replate medium (Product No. P748, O753) or BM-1 or BM-2.
3. Under aseptic conditions, transfer the shoots from the Petri dishes to the containers containing the fresh replate medium. The shoots should be placed about 1/4" to 1" apart on the medium.
4. Allow the shoots to continue to grow and develop. Root formation generally begins when the plantlets have 2-3 leaves. Continue to transfer the plantlets to fresh media every 30-60 days, increasing the spacing between the plants with each transfer. When the container is ready for transfer to a community pot in the greenhouse, most containers will have 15 to 25 plants depending upon the species.
5. Transfer the plants into a community pot using a finely ground orchid mix.

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:

6. 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.
7. While stirring the water, add the powdered medium and stir until completely dissolved.
8. 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.
9. Add agar while stirring; it will not dissolve but should disperse into a uniform suspension.
10. 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.
11. Add additional tissue culture grade water to bring the medium to the final volume.
12. 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 (muratic) acid to lower the pH of the medium.
13. While stirring, heat the solution to nearly boiling to melt the agar in the medium.
14. 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 <u>cannot</u> 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.

15. Sterilize the medium in a validated autoclave or pressure cooker at 1 kg/cm^2, 121 °C (15 psi, 250 ° F), for the time period described under "Sterilization of Media" below.
16. 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/cm^2 (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.

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	Orchid Maintenance/ Replate Medium	BM-1 Terrestrial Orchid Medium	BM-2 Terrestrial Orchid Medium	Orchid Multiplication Medium
COMPONENT	**P748**	**B141**	**B142**	**O753**
Ammonium Nitrate	825	-	-	825
Boric Acid	3.1	10	10	3.1
Calcium Chloride, Anhydrous	166	-	-	166
Cobalt Chloride·$6H_2o$	0.0125	0.025	0.025	0.0125
Cupric Sulfate·$5H_2O$	0.0125	0.025	0.025	0.0125
Na2 EDTA	37.3	37.3	37.3	37.3
Ferrous Sulfate·$7H_2O$	27.8	27.8	27.8	27.8
Magnesium Sulfate	90.4	100	100	90.4
Manganese Sulfate·H2O	8.5	25	25	8.5
Molybdic Acid (Sodium Salt)·2H2O	0.125	0.25	0.25	0.125
Potassium Iodide	0.42	-	-	0.42
Potassium Nitrate	950	-	-	950
Potassium Phosphate, Monobasic	85	300	300	85
Zinc Sulfate·7H2O	5.3	10	10	5.3
ORGANICS				
Activate Charcoal	2,000	-	-	-
Agar	7,000	5,000	6,000	7,000
Banana Powder	30,000	-	-	30,000
6-Benzylaminopurine (BA)	-	-	0.2	2
D-Biotin	-	0.05	0.05	-
Casein, enzymatic hydrolysate	-	500	500	-
Folic Acid	-	0.5	0.5	-
L-Glutamine	-	100	100	-
Glycine (Free Base)	-	2	2	-
MES (Free Acid)	1,000	-	-	1,000
myo-Inositol	100	100	100	100
α-Naphthaleneacetic Acid	-	-	-	0.5
Nicotinic Acid	1	5	5	0.5
Peptone	2,000	-	-	2,000
Pyridoxine·HCl	1	0.5	0.5	0.5
Sucrose	20,000	20,000	2,0000	20,000
Thiamine·HCI	10	0.5	0.5	10
Grams of powder to prepare 1 liter	64.31	26.22	27.22	32.20
pH ± 0.5 at RT	5.0	5.5	5.5	5.3

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 ☑
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐

Instructions/ Comments:

_____

_____

_____

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_____

_____

Species/Tissue Cultured: _____

NOTES

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