TERRESTRIAL ORCHID SEED SOWING KIT

Product No. O788

***Phyto*Technology 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
S008	Spatula/Transfer Tool, Round	1
P959/P334 – 1 roll	pH Strips	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
K425 – 1L	Knudson Modified Plus Replate Medium	2
T849 – 1L	Terrestrial (Cypripedium) Orchid Medium + w/o Casein	2
T839 – 1L	Terrestrial (Cypripedium) Orchid Medium + Casein w/o NH_4NO_3	2
B141 – 1L	BM-1 Orchid medium	2
B142 – 1L	BM-2 Orchid medium	2
M551 – 1L	Malmgren’s Modified Orchid Medium	2

MATERIALS REQUIRED BUT NOT PROVIDED

1. Beakers/containers: three 500-mL and one 250 mL
2. Media preparation container
3. Tissue culture grade water (Product No. W783 is sterile deionized water)
4. Commercial chlorine bleach (identified as 100% in text)
5. 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. P720)
6. 70% Isopropyl alcohol
7. Bunsen or alcohol burner (Product No. B966 or B876, respectively)

INTRODUCTION

*Phyto*Technology Laboratories' Terrestrial Orchid Seed Sowing Kit is designed to allow the user the flexibility of sowing a variety of terrestrial orchid species and hybrids. Included are seed sowing media (B141, M551, T839, T849) replate media (B141, M551, K425, T839, T849), and a multiplication/replate medium (B142) which enables the user to evaluate different media with the goal of determining the best ones for their species or hybrid of interest. 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.

SEED DISINFECTION

Orchid seeds are very small and contain very little or no food reserves. A single seed capsule may contain 1,500 to 3,000,000 seeds. Sowing the seed in vitro makes it possible to germinate immature seed (green capsule). It is much easier to disinfect the green capsule than individual seed after the capsule has split open. Lucke (1971) indicated that orchid seed can be disinfected when the capsule is about two-thirds ripe. Listed below is the estimated normal ripening times of capsules for various orchid genera (Lucke, 1971).

Orchid Genera	Time to Maturity [months]	Orchid Genera	Time to Maturity [months]
Bulbophyllum	3	Laelia	9
Calanthe	4	Masdevallia	3.5
Cattleya	11	Miltonia	9
Coelogyne	13	Odontoglossum	7
Cymbidium	10	Paphiopedilum	10
Cppripedium	3.2	Phalaenopsis	6
Dendrobium	12	Stanhopea	7
Epidendrum	3.5	Vanda	20

GREEN CAPSULE DISINFECTION

1. Soak the green seed capsule in 100% bleach solution for 30 minutes.
2. Dip the capsule in isopropyl alcohol or ethanol for 5-10 seconds. Remove the capsule from the alcohol and carefully flame off the excess alcohol.
3. Under aseptic conditions, using a sterile knife or scalpel, open the capsule and scrape out the seeds.
4. Carefully layer the seeds over the surface of the seed sowing medium (Product No. B141, M551, T839, T849). Seal the Petri plates or containers.

DRY SEED DISNFECTION

1. Collect seed and place in a small flask, bottle or a shortened pipet, which has one end sealed with cotton. Seal the other end of the pipet with cotton once the seeds have been inserted.
2. Prepare a solution containing 5-10% commercial bleach containing a few drops of Tween 20 (Product No. P720)
3. Add the bleach solution to the flask or draw up the solution into the pipet. Swirl the flask containing the seeds and bleach or repeatedly draw and aspirate the bleach solution in and out of the pipet.
4. Incubate the seeds in the manner described in Step 3 for 5-10 minutes.
5. Remove the bleach solution and rinse the seeds with sterile tissue culture grade water (Product No. W783).
6. Transfer the seeds to sterile seed sowing medium (Product No. B141, M551, T839, T849). Seal the Petri plates or containers.

REPLATING SEEDLINGS

1. It may take anywhere from 1 month up to 12 months for the seed to begin to germinate. Approximately 30 to 60 days after germination begins, it will be necessary to transfer the seedlings to fresh media for continued growth.
2. Prepare orchid maintenance/replate medium (Product No. B141, B142, M551, T839, T849).
3. Under aseptic conditions, transfer the seedling from the Petri dishes to the containers containing the fresh replate medium. Seedlings should be spaced about 1/4" apart on the medium.
4. Allow the seedlings to continue to grow and develop. Root formation generally begins when the plants have 2-3 leaves. Continue to transfer the seedlings 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.

REFERENCES

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

Lucke, E. 1971. Zur Samenkeimung Mediterraner Ophrys. Die Orchidee 22:62-65.

MEDIA FORMULATIONS

All components express in mg/L	BM-1 Orchid Medium	BM-2 Orchid Medium	Terrestrial (Cypripedium) Orchid Medium	Terrestrial (Cypripedium) Orchid Medium	Malmgren's Mod. Terrestrial Orchid Medium
COMPONENT	**B141**	**B142**	**T839**	**T849**	**M551**
Ammonium Citrate			19.0	19.0	
Ammonium Nitrate				1400	
Boric Acid	10.0	10.0	0.5	0.5	
Calcium Nitrate			400	400	
Calcium Phosphate, Tribasic					75.0
Cobalt Chloride·$6H_2o$	0.025	0.025			
Cupric Sulfate·$5H_2O$	0.025	0.025	0.025	0.025	
Na2 EDTA	37.25	37.25			37.26
Ferric Ammonium Citrate			25	25	
Ferrous Sulfate·$7H_2O$	27.85	27.85			27.8
Magnesium Sulfate, anhydrous	100	100	97.69	97.69	97.69
Manganese Sulfate·H_2O	25	25	1.54	1.54	1.54
Molybdic Acid (Sodium Salt)·$2H_2O$	0.25	0.25	0.02	0.02	
Potassium Chloride			100	100	
Potassium Iodide			0.1	0.1	
Potassium Nitrate			200	200	
Potassium Phosphate, Monobasic	300	300	200	200	75
Zinc Sulfate·$7H_2O$	10	10	0.5	0.5	
ORGANICS					
Activate Charcoal					1000
Agar	5000	6000	6000	6000	7000
6-Benzylaminopurine (BA)		0.2			
D-Biotin	0.05	0.05			0.05
Casein, Enzymatic Hydrolysate	500	500	400		400
Folic Acid	0.5	0.5			0.5
D-Glucose			20,000	20,000	
L-Glutamine	100	100			
Glycine	2.0	2.0			2.0
myo-Inositol	100	100			100
Nicotinic Acid	5.0	5.0			5.0
Pineapple Powder					20,000
Pyridoxine·HCl	0.5	0.5			5.0
Sucrose	20,000	20,000			
Thiamine·HCI	0.5	0.5			10.0
Grams of powder to prepare 1 liter	26.22	27.22	27.44	28.44	28.84
pH ± 0.5 at RT	5.5	5.5	5.5	5.5	4.25

*K425 is a proprietary formulation. Use K425 at 79.11 g/L.

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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_____

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Species/Tissue Cultured: _____

NOTES

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