FERN MULTIPLICATION KIT

Product No. F354

***Phyto*Technology Laboratories®**
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ISO 9001 REGISTERED FIRM

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KIT COMPONENTS

Product No.	Product Description	1 Each
	Box	1
	Instruction Manual	1
C215 – 10 ea	Culture Containers	1
F951 – 1 ea	Forceps, 8”	2
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
M401 – 1L	Murashige & Skoog Modified Basal Medium (w/ BA)	4
M508 – 1L	Murashige Fern Multiplication Medium	4
M555 – 1L	Murashige & Skoog Modified Multiplication Medium (w/ Kinetin)	4
S391 – 500 g	Sucrose	1
A296 (or A111) – 9 g	Agar	12
Optional at an additional charge	Fern Culture (NOT included in kits sent outside the continental USA)	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Beakers/containers: two 250-mL and one 500 mL
2. Media preparation container
3. 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. 720)
4. Tissue culture grade water (distilled/deionized, e.g., Product No. W783)
5. 70% Isopropyl alcohol
6. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
7. Fern runners (about 10 cm in length)
8. Murashige and Skoog (MS) Basal Medium (Product No. M519) for rooting
9. Indole-3-butyric acid, water-soluble salt (Product No. I560) to induce rooting. Add to 1/2 strength M519 medium at 1 mg/mL.

INTRODUCTION

Ferns are generally very easy to propagate through plant tissue culture. For this reason, most ferns that are commercially available through garden centers have been produced by this procedure (termed "micropropagation"). Many ferns can be started by taking the runner tips from actively growing plants and culturing them on a medium containing a mixture of essential nutrients, growth regulators and a gelling or solidifying agent.

The purpose of this kit is to demonstrate vegetative propagation and the effects of the various nutritive media on fern shoot multiplication. An established fern culture is provided with the kit so that it may be subcultured to observe the rapid, *in vitro* production of plants. With this kit cultures can also be generated from runner tips (not included with this kit).

MICROPROPAGATION STAGES

Stage I — Culture initiated with runner tissue (explant) and growth begins.
Stage II — Multiplication phase where the plantlet multiplies forming numerous shoots.
Stage III — Typically the rooting phase where individual shoots are stimulated to form roots.

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 powder 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 30 g/L of sucrose to M508; it is already contained in M401 and M555. Aside from these supplements, the media provided in this kit are complete and typically do not require other supplements.

5. Add 6-9 g/L of agar to all media. Add agar while stirring; it will not dissolve but should disperse into a uniform suspension.
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 fern. 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.
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/cm^2, 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/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.

CULTURE PROCEDURES

Establishing Fern Cultures

1. Wipe down all surfaces of the transfer hood or work area with 70% isopropyl alcohol. If using a hood, allow it to run for 15 min before beginning transfer operations. Place all the materials listed in the previous sections in the hood/work area. Place scalpels and forceps in a 250-mL beaker containing about 150 mL of 70% isopropyl alcohol.
2. Rinse the runners under running water then cut into 2.5 cm sections and transfer them to a 500-mL beaker. Place the beaker under the hood and pour the chlorine bleach solution over the runners, make certain all surfaces are properly covered. Leave the runners in the sterilization solution for 10 to 15 minutes and then pour off the solution. Rinse the runners three times in sterile distilled water with each rinse lasting approximately 1 min.
3. Place the culture vessels containing the media in the hood/work area.
4. All tools which now contact the tissue should be sterilized in alcohol and then flamed to remove any alcohol.
5. Take the sterilized fern runners and place them one at a time on a sterile Petri dish with sterile forceps.
6. Remove 1/4" from the ends of the runners by cutting with a sterile scalpel.
7. Place 2-4 runners/container on the culture medium surface.
8. Once all cultures have been completed, place them in low light (e.g., fluorescent light) at 25 ºC.
9. Explants in Stage I may turn black or brown if they were exposed to too much sterilization. If all cultures turn brown, repeat the sterilization procedures but reduce the time in the sterilization solution by 1/3. Within 15-30 days you should begin to see new growth coming from the runners that were not damaged by the sterilization.
10. After about 45 days, transfer the ferns to fresh media. To increase the number of plants, transfer to Stage II media. To root the ferns and prepare them for transferring to soil, use Stage III media.
11. Cultures in active Stage II should be transferred to fresh media every 30 days.

Subculture of Stage II

1. Use sterile forceps and remove the fern from the container and place it on a sterile Petri dish.
2. Carefully pull or cut the mass of multiple fern shoot apart into individual shoots.
3. Place 2-3 individual shoots into containers of fresh media.
4. Place the containers under low light at 25° C. Some of the shoots can be put on rooting medium or in potting soil to root.

APPROXIMATE SCHEDULE

Event	Timing
Isolation of fresh explants	Day 0
First appearance of micro-shoots (organogenesis)	Day 15
Noticeable shoot formation	Day 30
First subculture	Day 60
Transfer to rooting medium	Day 60+ (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.

Murashige, T and F Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

MEDIA FORMULATIONS

All components express in mg/L	Murashige & Skoog Modified Basal Medium (w/ BA)	Murashige Fern Multiplication Medium	Murashige & Skoog Modified Multiplication Medium w/Kinetin
COMPONENT	**M401**	**M508**	**M555**
Ammonium Nitrate	1650	1650	1650
Boric Acid	6.2	6.2	6.2
Calcium Chloride, Anhydrous	332.2	333	332.2
Cobalt Chloride·$6H_2o$	0.025	0.025	0.025
Cupric Sulfate·$5H_2O$	0.025	0.025	0.025
Na2 EDTA·$2H_2O$	37.26	-	37.26
Ferric Sodium EDTA	-	36.7	-
Ferrous Sulfate·$7H_2O$	27.8	-	27.6
Magnesium Sulfate	180.7	181	180.7
Manganese Sulfate·H2O	16.9	16.9	16.9
Molybdic Acid (Sodium Salt)·2H2O	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	0.83
Potassium Nitrate	1900	1900	1900
Potassium Phosphate, Monobasic	170	170	170
Sodium Phosphate Monobasic	-	255	148
Zinc Sulfate·7H2O	8.6	8.6	8.6
Adenine Hemisulfate	-	-	80
6-Benzylaminopurine (BA)	1	-	-
Glycine (Free Base)	2	-	-
Kinetin	-	2	1
myo-Inositol	100	100	100
a-Naphthaleneacetic Acid	0.1	0.1	0.1
Nicotinic Acid (Free Acid)	0.5	-	-
Pyridoxine·HCI	0.5	-	-
Sucrose	30000	-	30000
Thiamine·HCI	0.4	0.4	0.4
Grams of powder to prepare 1 liter	34.44	4.66	34.66

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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