LIFE TECH

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Your Molecular & Cell Technology Partner

FERN MULTIPLICATION KIT

Product No. F354

India Contact

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Kit Components

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	Box	1
	Instruction Manual	1
C913/C215-10ea	Culture Containers	1
F951-1ea	Forceps, 8”	2
S963-1ea	Scalpel Handle, No. 3	1
S971	Scalpel Blades	2
P334-1 Roll	pH Strips, 4.5-7.5	1
D940-10ea	Petri Dishes	1
V886	Vinegar (100 mL)	1
S803-100g	Sodium Bicarbonate (Baking Soda)	1
P068	Pipet, 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
S391-500g	Sucrose	1
A296-9g	Agar	12
	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. 1000 ml of sterile distilled water (Product No. W783)
5. 150 ml of 95% ethanol
6. 70% Isopropyl alcohol
7. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
8. Fern runners (about 10 cm in length)
9. MS Basal Medium M519 for rooting.
9. Indole-3-butyric acid, water-soluble salt (Product No. I560) to induce rooting. Add to ½ strength M519 medium at 1 mg/mL.

Introduction

Ferns are generally very easy to propagate through plant tissue culture. Because of this, most ferns that are commercially available through garden centers have been produced by this procedure (termed "micro-propagation"). 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 States

Stage 1 — Culture initiated with runner tissue (explant) and growth begins.
Stage II — Multiplication phase where the explant 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 2-6° 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 affect shelf life and product stability. 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. Add 6-9 g/L of agar to all media. Add 30 g/L of sucrose to M508; it is already contained in M401 and M555. Add any additionally desired heat stable supplements, such as PPM (Product No. P820) at 2 ml/L of medium. Aside from these supplements, the media provided in this kit are complete and typically do not require other supplements.
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 African violets. 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:
   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. The culture vessels (Product No. C913) are autoclavable. Media should be dispensed in these vessels prior to sterilization in an autoclave or pressure cooker. The lids of these culture vessels C093/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. Recently, the use of the microwave has also been shown to be successful at sterilizing media. 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. Times for sterilizing in a microwave are based on using a 1000-watt microwave with a turntable for more even distribution of heat. The times required for sterilization may vary depending upon the model of the microwave, power wattage, and the number of vessels in the microwave.

Media Sterilization Time

Volume of Medium per Vessel (mL)	Minimum Autoclaving[a] Time (min.)	Minimum Microwaving[b] Time (min.)
25	15-20	4-6
50	25	6-8
100	28	8-10
250	31	10-12
1000	40	NR
2000	48	NR
4000	63	NR

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

[b] Minimum Microwaving Time includes the time required for the liquid volume to reach a temperature of 121° C and remain at this temperature for a period of 3-4 minutes. Media used in this study contained 1.0 mL/L of PPM. Validation with your microwave is recommended. NR = Not Recommended

Culture Procedure

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 95% ethanol.

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 Clorox solution over the runners, making 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 ¼" 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 25º C.
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.

Schedule

Event	Timing
Isolation & culture of fresh explants	Day 0
First appearance of micro-shoots (organogenesis)	Day 15 (approximate)
Noticeable shoot formation	Day 30 (approximate)
First subculture	Day 45-60 (approximate)
Transfer to rooting medium	Day 60+ (When plantlets are large enough to handle)

Subculture of Stage II

1. Using sterile forceps, 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.

Media Formulation

All components expressed 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	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□H_2O	16.9	16.9	16.9
Molybdic Acid (Sodium Salt)□2H_2O	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□7H_2O	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□HCl	0.5		
Sucrose	30,000		30,000
Thiamine□HCl	0.4	0.4	0.4
Grams of powder to prepare 1 liter	34.44	4.66	34.66

Notes:

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