Cape Sundew/Venus Flytrap Tissue Culture Kit

Product No. C1835

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Table of Contents

Kit Components		
Product No.	**Product Description**	**Quantity**
	Box	1
	Instruction Manual	1
C215 – 10 ea	Culture Containers	1
C206 – 1L	CapeSundew/VenusFlytrap Multiplication Basal Medium	5
C216 – 1L	CapeSundew/VenusFlytrap Pretransplant Basal Medium	5
S391 – 500 g	Sucrose	1
A111/A296 – 9 g	Agar	10
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 ea
V886 – 15 mL	Vinegar	1
S803 – 25 g	Sodium Bicarbonate (Baking Soda)	1
P068	Pipet, Plastic Transfer	2
D940 – 20 ea	Petri Dishes	1
Optional at an additional charge	Cape Sundew or Venus Flytrap live culture (NOT included in kits sent outside the continental USA)	1

Materials Required But Not Provided

1. Murashige and Skoog (MS) medium with Vitamins (Product No. M519)
2. Beakers/containers: three 250-mL
3. Media preparation container
4. 10% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. P720)
5. Tissue culture grade water (distilled/ deionized, e.g., Product No. W783)
6. 70% Isopropyl alcohol
7. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
8. Cape Sundew or Venus Flytrap plant(s) – Actively growing with young shoots

Introduction

Carnivorous plants are predatory flowering plants with special trapping mechanisms that capture and kill prey such as insects, lizards, mice, spiders, small vertebrates, and etc., for nutrients. They utilized specialized leaves as traps with "bright colors, extra floral nectarines, guide hairs, and/or leaf extensions" to lure the prey. In addition to the specialized trap, the carnivorous plants also have a specialized mechanism to digest preys. Most carnivorous plants digest its prey by dissolving proteins or other compounds via acids and enzymes secreted through its gland; thus the nutrients become readily available for the plants to absorb. (Brittnacher, 2013)

The two most common types of carnivorous plants are Cape Sundew (*Drosera sp.)*and Venus flytrap (*Dionaea muscipula)*. Cape Sundew is a small rosette carnivorous plant. Its petioles are covered with green or red colored tentacles. When stimulated by a prey, the tentacles secrete mucilage and the petioles fold inward toward the center allowing more digestive glands to come in contact with the prey which help with the digestion process. (Yoko *et al*., 2013)

Similar to the Cape Sundew, the Venus flytrap's trapping mechanism is also specialized; however, it's more complex. Venus flytrap is also a small rosette plant with four or more leaves. The leaves are divided into two parts, a petiole with photosynthesis capability and a trap made up of a pair of terminal lobes hinged at the midrib. On the surface of each lobe are red anthocyanin pigments which attract pollinators and three hair-like trichomes which triggers the trap to close upon a prey's stimuli. Once a prey has been captured, the cilias along the edges of the lobes meshed together and mucilage is secreted from the edge of the lobes to prevent the prey from escaping, which leads to the digestion of prey for nutrients. (Volkov *et al.*, 2008)

PhytoTechnology Laboratories® Carnivorous Tissue Culture Kit (Product No. C1835) provides the necessary materials to initiate culture of Cape Sundew (*Drosera spatulata)* and Venus flytrap (*Dionaea muscipula)* from shoot tips. An established culture of Cape Sundew or Venus Flytrap is included in kit with culture purchase option. The purpose of this kit is to demonstrate the rapid growth of carnivorous plants *in vitro.*

Micropropagation Stages

Stage 1 – Culture initiated with seeds and growth begins.

Stage 2 – Multiplication phase where the explant multiplies to form numerous new shoots.

Stage 3 – 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 storage conditions. 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 sucrose to C206 and C216.
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. 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. 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 culture vessels C215 should not be tightly sealed during sterilization to allow for proper steam and pressure penetration.
9. Sterilize the medium in a validated autoclave or pressure cooker at 1kg/cm2, 121 °C (15 psi, 250 °F), for the time period described under “Sterilization of Media” below.
10. 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 Time

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 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.

Culture Procedure

Germination of Carnivorous Seeds

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 the hood/work area. Place scalpels and forceps in a 250-mL beaker containing about 150 mL of 70% isopropyl.
2. Place seeds into a small test tube with cap and add an appropriate amount of the 10% bleach-Tween solution to cover the seed completely.
3. Soak the seeds in the bleach-Tween solution for 8 to 10 min and then pour out the solution carefully as to not pour out the seeds.
4. Rinse the seeds three times in sterile distilled water with each rinse lasting approximately 5 min.
5. Carefully transfer the seed onto sterile half-strength MS medium with Vitamins. Once all the cultures have been completed, place them in a low light (e.g., fluorescent light) at 25 °C.
6. Once shoots have developed they can be subcultured (individually transferred) onto fresh medium for continued multiplication (Product No. C206) or subcultured onto fresh pre-transplant medium (Product No. C216) which later can be removed and planted in potting soil.
7. Be patient as many months of subculturing may be required to achieve rapid multiplication rates.

Establishment of Carnivorous Cultures from Isolated Shoot Tips *ex Vitro*

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 the hood/work area. Place scalpels and forceps in a 250-mL beaker containing about 150 mL of 70% isopropyl alcohol.
2. Select healthy plantlets and cut the petiole near the point where it attaches to the stem.
3. Rinse the leaves under running water then transfer the leaves to the 500-mL beakers.

4. Place the beaker under the hood and pour the 10% bleach-Tween solution over the leaves, making certain all leaf surfaces are properly covered.
5. Soak the leaves in the sterilization solution for 20 min (Cape Sundew) or 10 min (Venus Flytrap) and then pour off the solution.
6. Rinse the leaves three times in sterile distilled water with each rinse lasting approximately 10 min.
7. Transfer each sterilized leaf to a separate sterile Petri dish and remove the petiole with a sterile scalpel.
8. Remove any part of the tissue that turned white from the bleach. Section the remaining leaf area and petiole into small pieces.
9. Transfer one to four leaf or petiole sections to each culture vessel so that the abaxial (underside) side or the leaf or cut surface of the petiole touches the medium. Once all cultures have been completed, place them in low light (e.g., fluorescent light) at 25 °C.
10. Once shoots have developed they can be subcultured (individually transferred) onto fresh medium for continued multiplication (Product No. C206) or subcultured onto fresh pre-transplant medium (Product No. C216) which later can be removed and planted in potting soil.
11. Be patient as many months of subculturing may be required to achieve rapid multiplication rates.

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 seeds or shoot tips.
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 Cape Sundew or Venus Flytrap 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 or clumps.
5. Place these shoots onto fresh multiplication medium (Prod. No C206); 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 (approximate)
Isolation & culture of fresh explants	Day 0
Noticeable shoot formation	Day 30
First subculture	Day 45 – 60
Transfer to rooting medium	Day 60+ (When plantlets are large enough to handle)

Media Composition

All components express in mg/L	Cape Sundew/ Venus Flytrap Multiplication Basal Medium	Cape Sundew/ Venus Flytrap Pretransplant Basal Medium
Component	**C206**	**C216**
Ammonium Nitrate	400	825
Boric Acid	6.2	3.1
Calcium Chloride, Anhydrous	332.2	166.5
Cobalt Chloride·6H_2O	0.025	0.0125
Cupric Sulfate·5H_2O	0.025	0.0125
Ferric Sodium EDTA	-	18.35
Disodium EDTA·2H_2O	74.5	-
Ferrous Sulfate	55.7	-
Magnesium Sulfate, Anhydrous	180.7	90.5
Manganese Sulfate·H_2O	16.9	8.45
Molybdic Acid (Sodium Salt)·2H_2O	0.25	0.125
Potassium Iodide	-	0.415
Potassium Nitrate	480	950
Potassium Phosphate Monobasic	-	85
Sodium Phosphate Monobasic	380	-
Zinc Sulfate·7H_2O	8.6	4.3
Adenine Hemisulfate	80	-
6-γ,γ-Dimethylallylaminopurine (2IP)	1.0	-
myo-Inositol	100	50
Thiamine·HCl	0.4	0.2

References

Brittnacher, John. "What are Carnivorous Plants?". International Carnivorous Plant Society. Retrieved from http://www.carnivorousplants.org/cp/WhatAreCPs.php. 17 Dec 13

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

Volkov, Alexander G., Tejumade Adesina, Vladislav S. Markin, and Emil Jovanov. 2008. Kinetic and mechanism of *Dionaea muscipula* trap closing. *Plant Physiology*. 146(2):694-702.

Yoko Nakamura, Michael Reichelt, Veronika E. Mayer, and Axel Mithöfer. 2013. Jasmonates trigger prey-induced formation of 'outer stomach' in carnivorous sundew plants. *Proc R Soc B.* 280(1759): 2013.0228

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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