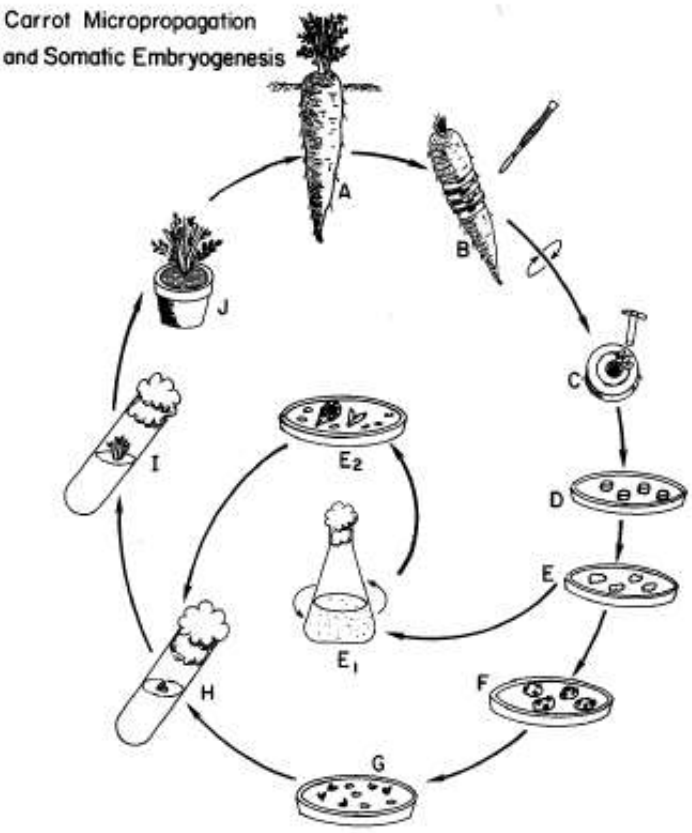
Carrot Tissue Culture Kit

Product No. C1955



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KIT COMPONENTS

Product No.	Product Description	1 Each
	Box	1
	Instruction Manual	1
C215 – 10 ea	Culture Containers	1
C212 – 1L	Carrot Callus Initiation Medium	5
C222 – 1L	Carrot Shoot Development Medium	5
S391 – 500 g	Sucrose	1
G434 – 2 g	Gellan Gum	10
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 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
C215 – 10 ea	Culture Containers	1
C212 – 1L	Carrot Callus Initiation Medium	5

MATERIALS REQUIRED BUT NOT PROVIDED

1. Sterile #2 cork borer and a glass or metal rod that fits it
2. 1000 mL beaker and 250 mL beaker
3. Beakers/containers: three 250-mL
4. Media preparation container
5. 20% chlorine bleach solution supplemented with a few drops of Tween-20 (Product No. P720)
6. 1000 mL of sterile distilled water (Product No. W783)
7. Tissue culture grade water (e.g., distilled/ deionized)
8. 70% Isopropyl alcohol (IPA)
9. Stir-plate
10. Magnetic Stir Bar (Product No. B015)
11. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
12. 1 or 2 healthy, undamaged, and regularly shaped carrot roots.

INTRODUCTION

Carrot [*Daucus carota* L. subsp. Sativus (Hoffm.)] is a cool season plant grown for its edible storage tap root. It is derived from the wild carrot (*D. carota* L. subsp. *carota*) or Queen Anne's Lace and belongs to the *Apiaceae* family. Studies of carrot tissue culture were first reported independently by Gautheret and Nobecourt in 1939 (Wiggans 1954). In 1958, the first study of carrot embryogenesis was reported by Steward *et al*. and Reinert via suspension and callus cultures, respectively (Torres, 1989). Since then, carrot tissue cultures have been studied by numerous researchers. It has become a popular model system for the study of somatic embryogenesis.

Somatic embryogenesis is an important pathway that is unique to the plant kingdom (Zimmerman, 1989). This process is useful and helpful for investigating and understanding many biochemicals, physiological, and genetic aspects of plant cell culture (Kiyosue et al., 1989, Torres, 1989, Zimmerman, 1993). Somatic embryos of carrot tissues can be easily be induced via changing the culturing conditions (Zimmerman, 1989), i.e., transfer carrot tissues from an extremely high concentration auxin-containing culture medium, such as 2,4-Dichlorophenozylacetic acid (2,4-D), to a non-auxin-containing culture medium (Kiyosue et al., 1989). Beside the culturing conditions, it is equally important to use cultures that are relatively young (i.e., a year or younger) as recommended by Zimmerman (1989).

A general overview of somatic embryogenesis has been described by Zimmerman (1993) are as followed:

1. The carrot callus cell lines are established from small hypocotyl pieces of germinated seeds.
2. The embryogenic cells of cultured cells are selected via sieving or gradient fractionation.
3. The selected culture cells are transferred to an auxin-free culture medium.
4. The cells are diluted to a relatively low density.

Other researchers such as Kiyosue *et al*. (1989) have reported the use of hypochlorite treatment on carrot seeds to be as effective in inducing somatic embryos. This may be due to the level of stress exerted upon the tissues similar to those of the high auxin concentration (Kiyosue *et al.*, 1989).

This kit contains *Phyto*Technology Laboratories® Carrot Callus Initiation Medium (Prod. No. C212) which contains the macro- and micronutrients, vitamins and plant growth regulator (such as 2,4-D) required to initiate carrot callus from root tissue and Carrot Shoot Development Basal Medium (Prod. No. C222) which contains the same nutrients and vitamins as Prod. No. C212, however, kinetin is used in place of 2,4-D for the support of embryo development. Additional supplements such as carbohydrate (e.g., D-Sucrose (Prod. No. S391)) and gelling agent (e.g., Gellan Gum (Prod. No. G434)) are also included to create a complete medium for the support of culture growth.

The purpose of this kit is to demonstrate Stage 1 and 2 of *in vitro* propagation through carrot callus initiation as well as Stage 3 in development of embryos obtained in stage 2. This kit will outline the method for establishing carrot callus cultures.

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 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 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 2 g/L of gellan gum while stirring; it will not dissolve but should disperse into a uniform suspension.
5. Add 30 g/L sucrose to C212 and C222.
6. Add additional tissue culture grade water to bring the medium to the final volume.
7. While stirring, determine the pH using the pH paper (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. Sterilize the medium in a validated autoclave or pressure cooker at 1.1 kg_f/cm^2, 121°C (16 psi, 251°F), for the time period described under "Sterilization of Media" below.
9. Allow the medium to cool for 15 minutes preferably while stirring.
10. Dispense the medium into the culture vessels under aseptic conditions 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 and should be autoclaved/pressure cooked prior to use at the 121°C.
11. Allow medium to gel for 3 hrs.

STERILIZATION OF MEDIA

Plant tissue culture media are generally sterilized by autoclaving at 121°C and 1.1 kg_f/cm^2 (16 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 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 PROCEDURES

Establishing Carrot Callus

1. Wipe down all surfaces of the transfer hood or work area with 70% IPA. 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% IPA.
2. Clean carrot root by scrubbing under running tap water to remove any surface soil. Trim the carrot into 100-mm sections and place them in a 1000-mL beaker. Cover with 20% Clorox solution for approximately 20 min and then decant the chlorine solution. Rinse the explant three times in sterile distilled water covering the tissue with each rinse.
3. Transfer sterilized carrot slices to a sterile Petri dish. Using a sterile cork borer, punch out 8-10 cylinders of tissue from the secondary phloem cambial region of the carrot slices. Do not punch out more than two cylinders of tissue at one time, as you may have difficulty in removing them from the cork borer. Using a sterile rod, push the cylinders from the cork borer into a sterile Petri dish. Using a sharp, sterile scalpel, remove the ends of the tissue cylinder. Next cut the remaining portion of the cylinder into 2- to 3-mm-thick sections and inoculate into the culture vessels containing the Carrot Callus Initiation Medium.
4. Repeat Step 3 until all culture vessels containing the Carrot Callus Initiation medium have been inoculated. Use a different forceps for each explant and flame the forceps between transfers.

Callus Maintenance/Shoot Development

1. Callus should be removed from the primary explant after 45 days. The calli can be subcultured onto the same medium for further callus growth or it can be subcultured onto the Carrot Shoot Development medium (Prod. No. C222) for shoot initiation.

APPROXIMATE SCHEDULE

Event	Timing
Isolation of fresh explants	Day 0
First subculture	Day 60 (approximate)
Isolation of callus	Day 120 (approximate)

LITERATURE CITED

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

Kiyosue, Tomohiro, Hiroshi Kamada, and Hiroshi Harada. 1989. Induction of somatic embryogenesis from carrot seeds by hypochlorite treatment. *Plant Tissue Culture Letters*. 6(3):138-143.

Torres, Kenneth C.. 1989. Establishment and maintenance of carrot callus. *Tissue Culture Techniques for Horticultural Crops*. Pp. 111-115. New York: AVI

Wiggans, Samuels C.. 1954. Growth and organ formation in callus tissues derived from *Daucus carota*. *American Journal of Botany*. 41(4):321-326.

Zimmerman, J. Lynn. 1993. Somatic embryogenesis: a model for early development in higher plants. *The Plant Cell*. 5:1411-1423.

MEDIA FORMULATIONS

All components are expressed in mg/L	Carrot Callus Initiation medium	Carrot Shoot Development Medium
Ammonium Sulfate	134	134
Boric Acid	3	2
Calcium Chloride, Anhydrous	113.24	113.24
Cobalt Chloride·6H2O	0.025	0.025
Cupric Sulfate·5H2O	0.025	0.025
Na2 EDTA·2H2O	37.3	37.3
Ferrous Sulfate·7H2O	27.8	27.8
Magnesium Sulfate, Anhydrous	122.09	122.09
Manganese Sulfate·H2O	10	10
Molybdic Acid (Sodium Salt)·2H2O	0.25	0.25
Potassium Iodide	0.75	0.75
Potassium Nitrate	2500	2500
Sodium Phosphate Monobasic	150	150
Zinc Sulfate·7H2O	2	2
Kinetin	-	0.2
2,4-Dichlorophenoxyacetic Acid	1	-
myo-Inositol	100	100
Nicotinic Acid (Free Acid)	1	1
Pyridoxine·HCl	1	1
Thiamine·HCl	10	10
Grams of powder to prepare 1L	3.21 g	3.21 g

STOCK SOLUTION/MEDIUM 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 ☑
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
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Instructions/ Comments: _____

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Species/Tissue Cultured: _____

NOTES

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