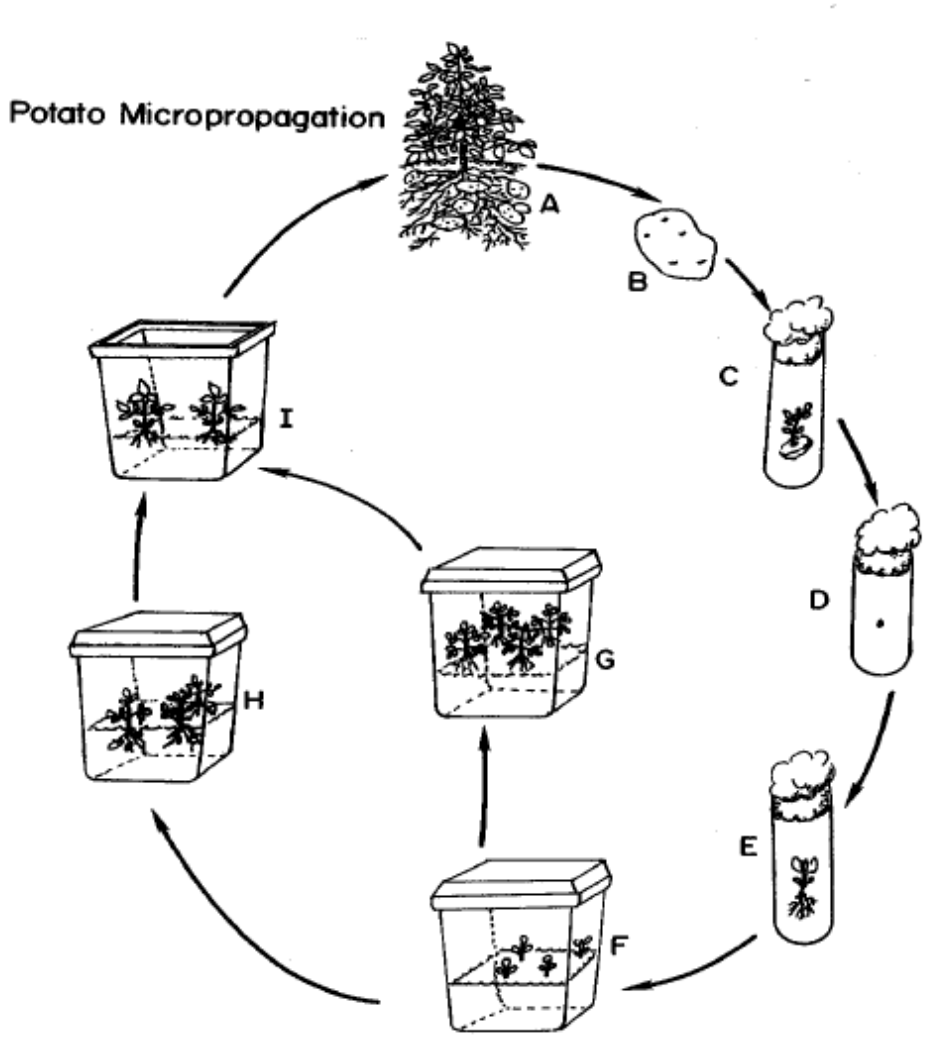
Potato Tissue Culture Kit

Product No. P6963



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KIT COMPONENTS

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	Box	1
	Instruction Manual	1
A111/A296 – 9 g	Agar	10
B130 – 100 mL	6-Benzylaminopurine (BA) Solution (1 mg/mL)	1
C215 – 10 ea	Culture Container	1
C186 – 10 g	Calcium Pantothenate	1
D940 – 20 ea	Petri Dishes	1
F951 – 1 ea	Forceps, 8”	2
G198 – 25 mL	Gibberilic Acid Solution (1 mg/mL)	1
M404 – 1L	Murashige & Skoog (MS) Medium with Gamborg Vitamin	5
M516 – 1L	Murashige & Skoog (MS) Modified BC Potato Medium	5
N605 – 100 mL	NAA Solution (1 mg/mL)	1
P334 – 1 roll	pH Strips, 4.5 – 7.5	1
S391 – 500 g	Sucrose	1
S963 – 1 ea	Scalpel Handle, No. 3	1
S971	Scalpel Blades, No. 11	2
S803 – 25 g	Sodium Bicarbonate (Baking Soda)	1
V886 – 15 mL	Vinegar	1

MATERIALS REQUIRED BUT NOT PROVIDED

1. 2 small-mouth Mason jars
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. Tissue culture grade water (distilled/deionized, e.g., Product No. W783)
7. 70% Isopropyl alcohol
8. Bunsen or alcohol burner (Product No. B966 or B876, respectively)
9. Serological Pipettes: 2 mL (Product No. P992) or 5 mL (Product No. P993)
10. Waterproof marking pen and labels
11. Two (2) healthy medium-sized potatoes.

INTRODUCTION

Potato, *Solanum tuberosum* L., is one of the most important non-cereal crops in the world. Potato production ranks fourth worldwide after rice (*Oryza sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) (Aryakia and Hamidoghi, 2010). Conventionally, potato is propagated vegetatively in which tubers are used as planting materials; however, this method is prone to infection by pathogens which can result in poor quality tubers. Propagation of potato through micropropagation techniques yields many benefits in comparison to the conventional method. Micropropagation techniques such as clonal propagation of nodal segments and microtuberization yields high quality and pathogen-free potato plants and miniaturized tuber seeds, also known as microtubers.

A number of different approaches have been successfully used in the generation of potato plants from *in vitro* cultures. Roest and Bokelman (1969) obtained plantlet generation from potato stem segments when explants were cultured on a Murashige and Skoog (MS) medium supplemented with 10 mg/L GA_3, 1.0 mg/L benzylaminopurine (BA), and 1.0 mg/L indole-3-acetic acid (IAA). Espinoza *et al.* (1984) have reported on the micropropagation of potato by either nodal section or shake cultures. They found that when nodal sections were inoculated onto a MS culture medium supplemented with 0.25 mg/L GA_3 and 2.0 mg/L calcium pantothenic acid, the number of nodes increased six fold within 3-4 weeks. When nodal sections were cultured on a liquid MS medium supplemented with 0.4 mg/L GA_3, 0.5 mg/L BA, 0.01 mg/L NAA, 2.0 mg/L calcium pantothenic acid, and 2% sucrose, there was a 10- to 20-fold increase in the number of nodes in 2-3 weeks.

Microtuberization is another technique for micropropagation of potato in which an established potato culture can be induced with high levels of sucrose to produce microtubers. It has been reported by Kanwal *et al.* (2006) that 96.6% of potato cultures of cultivar Kuroda produced microtubers when transferred to standard MS medium supplemented with 8% sucrose, while the same cultivar produced 0% microtubers when transferred to the same basal medium supplemented with 3% sucrose. Kanwal *et al.* (2006) also reported that MS medium supplemented with 0.75 mg/L BA and 8% sucrose showed maximum microtuberization of 6.33 per explant. Aryakia and Hamidoghi (2010) have also reported microtuber induction success with 0.75 mg/L of BA for

cultivars Arinda and Diamant. Moreover, other researchers have also reported success with inducing potato microtubers on MS medium supplemented with high concentrations of sucrose. Rafique *et al*. (2004) have reported maximum microtubers induced for potato cultivars Santa, Cardinal, Diamant and Desiree when cultured on MS medium supplemented with 1 µM BA and 6% sucrose.

The purpose of this kit is to demonstrate the rapid growth of potato node segments and formation of microtubers *in vitro.* This kit provides the necessary materials to initiate cultures from potato sprouts.

POTATO MICROPROPAGATION MEDIA RECOMMENDATIONS

Components are used at (g/L) or (mL/L)	Multiplication Method		Microtuberization Method
Base Medium M516 or M404	M516 – BC Potato medium	M404 – MS Medium	M404 – MS Medium
	4.41	4.44	4.44
S391 – Sucrose	20	20	80
B130 – BA Solution		0.5 mL	0.75 mL
N605 – NAA Solution	0.01 mL	0.1 mL	
G198 – Gibberellic solution	2.5 mL	2.5 mL	
C186 – Calcium Pantothenate Acid	0.002	0.002	
A111 – Agar	6	6	8
Final pH Desire	5.6 – 5.8	5.6 – 5.8	5.6 – 5.8

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 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 all the heat stable components, e.g., Sucrose, BA and NAA.
5. 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 meter. 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 (muriatic) acid to lower the pH of the medium.

8. Dispense the medium into the culture vessels (Product No. C215) before (or after) autoclaving according to your application. Add all non-liable components, e.g., GA_3 and Calcium Pantothenate Acid, after autoclaving via a sterile syringe filter. Swirl the media bottle to mix the components into the prepared media.
9. 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.
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 PROCEDURES

Establishing potato cultures

1. Place the basal end of a medium-sized potato in a Mason jar filled with water; repeat with the second potato. Allow several weeks for the sprouting of eyes to occur.
2. 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.
3. Remove sprouts and sterilize them for 10 min in 20% chlorine bleach solution and then rinse the tissues three times with sterile distilled water covering the tissues with each rinse.

4. Aseptically transfer the sterilized potato sprouts on to a sterile Petri dish and section the sprouts into 10-mm sections with each section containing one node. Inoculate four to five nodes per culture vessels containing the multiplication medium. Incubate cultures under low-light conditions at 25 °C.
5. Three weeks after inoculation, remove shoots from the culture vessels, divide into sections containing three to four nodes, and position the nodal segments horizontally onto fresh multiplication medium as described in the previous step. Repeat this procedure every 3 weeks.

Microtuberization of potato 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.
2. Aseptically remove nodal segments containing 3 to 4 nodes each from an actively growing potato culture. Transfer 3 to 4 nodal segments per culture container vertically onto culture vessels containing the microtuberization medium.
3. Incubate cultures under low-light conditions at 25 °C.

APPROXIMATE SCHEDULE

Event	Timing (approximate)
Initiation of cultures on multiplication medium	Day 0
Subculture of plantlets on multiplication medium	30 to 60 days
Microtubers formation	10 weeks

MEDIA FORMULATIONS

Components are expressed in mg/L	M516 – BC Potato Medium	M404 – MS medium
Ammonium Nitrate	1650	1650
Boric Acid	6.2	6.2
Calcium Chloride, Anhydrous	333	332.2
Cobalt Chloride•$6H_2O$	0.025	0.025
Cupric Sulfate•$5H_2O$	0.025	0.025
Ferric Sodium EDTA	36.7	-
Sodium EDTA	-	37.26
Ferrous Sulfate	-	27.8
Magnesium Sulfate, Anhydrous	181	180.7
Manganese Sulfate•H_2O	16.9	16.9
Molybdic Acid (Sodium Salt)• $2H_2O$	0.25	0.25
Potassium Iodide	0.83	0.83
Potassium Nitrate	1900	1900
Potassium Phosphate, Monobasic	170	170
Zinc Sulfate•$7H_2O$	8.6	8.6
myo-Inositol	100	100
Nicotinic Acid (Free Acid)	0.5	1
Pyridoxine•HCl	0.5	1
Thiamine•HCl	0.4	10
Kinetin	0.04	-
Glycine	2	-

REFERENCES

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Rafique, Tariq, M. Jafar Jaskani, Hasnain Raza and Mazhar Abbas. 2004. *In vitro* studies on microtuber induction in potato. *International Journal of Agriculture & Biology*. 6(2):375-377.

Roest, S. and G.S. Bokelmann. 1976. Vegetative propagation of *Solanum tuberosum* L. Potato Res. 19:173.

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 ☑
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐
					☐

Instructions/ Comments:

_____

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Species/Tissue Cultured: _____

NOTES

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