Plant Surface Sterilization Kit

Rapid Sterilization of Plant Seeds and Tissues for in vitro Growth

Revision # 5100-200-3G01

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Application Manual

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Catalog # 5100-200
50 Samples

Storage temperature:
Ambient temperature (15–30°C)

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1. Introduction

Plant tissue culture, also known as in vitro plant culture or micro-propagation, is the growth or maintenance of plant cells, tissues, or whole plants in vitro using a defined growth medium. Plastic and glass vessels are used for solid and liquid culture to maintain plant cells, seeds, or tissues in a sterile environment.

The Plant Surface Sterilization Kit is designed to sterilize seeds for germination and plant tissues for callus induction without affecting the ability of the seed or tissue to grow in culture or, at a later time, in soil. The two solutions in this kit preserve plant tissue characteristics for successful growth while providing surface sterilization to remove contaminating microorganisms associated with plant seeds and plant tissues (explants) prior to in vitro incubation using sterile growth medium.

The following protocol has been developed as a standard guideline for surface sterilization and will work on a wide variety of seed and explant types. Specific seeds and tissues may require modification to this basic protocol. For example, larger plant seed and tissue samples often require longer sterilization time. Storage plant tissues (tubers) may need to be peeled and cored before sterilization and prior to plating on culture media.

2. Kit Components and User Supplied Materials

2.1 Plant Surface Sterilization Kit Components

Description	Quantity
Sterilization Solution A	1 x 250 ml bottle
Sterilization Solution B	1 x 250 ml bottle
Reusable Polypropylene Container for Large Plant Samples	1 each
Reusable Polypropylene Container for Small Plant Samples	1 each
Dissecting Forceps	1 each
User Manual	1 each
MSDS	1 each
Certificate of Analysis	1 each

2.2 User Supplied Materials

Plant Tissue Culture Medium (See Section 8, Related Products)
Appropriate Tissue Culture Plates or Boxes (see Section 8, Related Products)
Appropriate Plant Growth Regulators
Sterile Deionized or Distilled Water (autoclaved or 0.2 µm filtered)
Sterile Environment (laminar flow culture hood)

3. Safety Precautions

Sterilization Solutions A and B contain components that may cause irritation or burning when in contact with human tissue or during inhalation. Wear personal protective equipment to prevent skin contact (e.g. gloves, lab coat, and eye protection) and prevent inhalation of reagent vapors and consumption of liquid during use. Consult the enclosed Material Safety Data Sheet for additional details.

4. Detailed Protocol for Seed and Explant Sterilization

NOTE: Recommended incubation times are used for the majority of applications and may be adapted to meet individual requirements.

NOTE: The size and specific type of explant tissue necessary for in vitro culture varies by plant and by overall experimental procedure, and is not described in this protocol.

1. If seeds or explant contains obvious dirt or other contaminants, remove them by rinsing with water.
   NOTE: If necessary, a mild soapy detergent may be used to remove dirt and/or contaminants. Thoroughly rinse seeds or explant with water to remove detergent before proceeding.

2. Place the seeds or explant in the small or large sterilization container provided in the kit.
   Choose the container which provides enough area for the sterilization solutions to cover the samples.
   NOTE: The Plant Surface Sterilization Kit is designed for batch processing seed and tissue samples. A pouch or filter bag is not required to hold the sample, but the solutions may be used with cheesecloth or other pouching methods if necessary. When using a pouch, ensure adequate contact occurs between the sample and the sterilization solutions during the process.
   NOTE: For very small seeds or explants, a smaller container such as a microcentrifuge tube can be used to contain the sample during processing.

3. Add enough Sterilization Solution A to submerge the sample.
   For most seed or explant samples, the volume will be approximately 5 ml. If using a microcentrifuge tube, a volume of 500 µl should be sufficient.

4. Incubate for 5 minutes at room temperature with occasional agitation.
   The closed container with the sample may be placed on a shaking platform with gentle rocking, or may be swirled or inverted by hand every few minutes.

5. Remove as much of the residual liquid as possible using a sterile pipet.
   The majority of the liquid can be decanted into a waste container, but use caution that the sample is not lost.

6. Add enough Sterilization Solution B to submerge the sample.
   For most seed or explant samples, the volume will be approximately 5 ml. If using a microcentrifuge tube, a volume of 500 µl should be sufficient.

7. Soak seeds or explant in Sterilization Solution B for 15 minutes with occasional agitation.
   The closed container with the sample may be placed on a shaking platform with gentle rocking, or may be swirled or inverted by hand every few minutes.

8. Remove as much of the residual liquid as possible using a sterile pipet.
   The majority of the liquid can be decanted into a waste container, but use caution that the sample is not lost.

9. Add enough sterile distilled water to submerge the sample.
   For most seed or explant samples, the volume will be approximately 5 ml. If using a microcentrifuge tube, a volume of 500 µl should be sufficient.

10. Incubate at room temperature for 5 minutes with occasional agitation.
    The closed container with the sample may be placed on a shaking platform with gentle rocking, or may be swirled or inverted by hand every few minutes.

11. Remove as much of the residual liquid as possible using a sterile pipet. Repeat steps 9-11 two more times.
    The sample is now ready for in vitro culture.

12. Flame-sterilize or autoclave the forceps provided in the kit.

13. Using the cooled, sterile forceps, transfer the seeds or explant tissue to an agar plate containing the appropriate growth media and growth regulators.
    Small seeds may be transferred in the water using a sterile transfer pipet.

14. Maintain cultures at approximately 25°C under low- to moderate-intensity light or follow plant-specific growth requirements.
    If possible, incubate plates at an angle sufficient to cause excess water to drain away from the sample.

15. Depending on the type of seeds or explant, germination will occur within approximately 1 to 4 weeks. For example, Alfalfa, Arabidopsis and California poppy will germinate in approximately one week; citrus will require approximately 2 - 4 weeks. Callus initiation will typically appear after 2 to 4 weeks.

    NOTE: If inducing callus formation on the agar plate, large sterilized seeds should be cut in half and large sterilized explants should be sliced prior to plating on culture media. Sterile instruments must be used at all times.

    NOTE: The germination period for seeds or explants that have been stored may be longer than the time needed by fresh seeds. Factors such as the sample age, storage conditions, type, and pre-harvest factors will contribute to the time required for germination and the overall success rate of the experiment. Seed germination efficiency is typically less than 100%.

16. Examine cultures every 3-5 days for contamination.
    Slick or shiny areas on the aqar are typically bacterial contamination while fuzzy areas are typically due to fungal contamination. Do not open containers that are contaminated: Seal lid and plate together using Parafilm™ or tape and decontaminate by autoclaving for at least 15 minutes at 15 psi. Discard appropriately. See Section 5.2 for troubleshooting contamination problems.

5. Troubleshooting

5.1 No Germination or Slow Germination

Ensure that sterilized seed or tissue is rinsed thoroughly with sterile water prior to placing on growth media.

For small seeds (Arabidopsis or Orchid) or fragile organs (anther, stamen, pistil), the incubation time in Sterilization Solution B may be reduced from 15 minutes to 5 - 10 minutes.

Sixteen hours of fluorescent light and 8 hours of darkness are recommended as starting conditions for most plants. Adjust light exposure as needed to optimize germination.

5.2 Bacterial and Fungal Contamination

Successful control of contamination depends largely upon strict adherence to aseptic techniques. By observing where on a plate contamination arises, it may be possible to determine its source. For example, contamination not associated with the sample suggests potential airborne or culture media sources. Aseptic techniques include appropriate covering of hands and clothing; and ensuring that nothing unnecessary moves near or disturbs open or partially uncovered samples or plates.

An approved laminar flow hood with ultraviolet decontamination is recommended for all sterilizing and culturing activities. Treating the work area with 70% ethanol, 10% bleach, or other sterilization solutions prior to use is recommended. Additional decontamination preventive measures may also include sterilizing the work area floor with a decontaminating agent, keeping doors and windows closed during the procedure and filtering (0.2 µm) air entering the work area.

A sterile work surface (e.g. sterile Petri dish or culture plate) is recommended as a surface on which to trim sterilized tissue prior to culturing.

Although antibiotics are used routinely in animal cell cultures, they are not widely used in plant tissue cultures because they are often ineffective, kill the culture, or induce chromosomal instability. Plants may metabolize antibiotics or their degradation products with unpredictable results. If antibiotic usage is deemed necessary, the agents of choice are those that act specifically within bacterial cell walls and bacterial membranes. Selection of an effective agent cannot be made until the contaminants are known.

6. Recommended Reference Format for Publications

Specific seed or tissue explant was sterilized prior to in vitro culture using the Plant Surface Sterilization Kit (Qbiogene, Inc., CA).

7. References

1. *Chu, Z. Q, et al (1975) Chinese Academie Sinica, 5 : 484-490.*
2. *Dodds, John H. and Lorin W. Roberts. Experiments in Plant Tissue Culture, 3rd Ed. Cambridge University Press, 1995.*
3. *Gamborg, O.L., R.A. Miller and K. Ojima. (1968) Nutrient Requirements of Suspension Cultures of Soybean Root Cells. Experimental Cell Research 50: 151-158.*
4. *Gamborg, O. L., T. Murashige, T.A. Thorpe and I. K. Vasil. (1976). Plant Tissue Culture Media. In vitro 12(7): 473 – 478.*
5. *Murashige, T. and F. Skoog. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum, 15: 473 – 497.*
5. *Sharp, W. R., D. A. Evans, P. V. Ammirato and Y. Yamada Eds. Handbook of Plant Cell Culture, (2) 198. Macmillan Publishing Company, 1984.*

8. Related Products

Cat. #	Description & Size	Format
Growth Media		
5100-035	Murashige & Skoog Basal Salts, 10L Pouch	Powder
5100-014	Murashige & Skoog Basal Salts, 10X Liquid, 500ml	Liquid
5100-335	Murashige & Skoog Basal Medium (Salts, Vitamins), 10L Pouch	Powder
5100-125	Murashige & Skoog Complete Medium (Salts, Vitamins, Sucrose), 1L Pouch	Powder
5100-114	Murashige & Skoog Complete Medium (Salts, Vitamins, Sucrose), Liquid, 500ml	Liquid
5100-225	Murashige & Skoog Complete Agar (Salts, Vitamins, Sucrose, Agar), 1L Pouch	Powder
5100-042	Murashige & Skoog Vitamins Powder, 5g	Powder
5100-044	Murashige & Skoog Vitamins (1000X) Liquid, 20ml	Liquid
5101-035	Gamborg's B5 Basal Salts, 10L Pouch	Powder
5101-014	Gamborg's B5 Basal Salts, 10X Liquid, 500ml	Liquid
5101-335	Gamborg's B5 Basal Medium (Salts, Vitamins), 10L Pouch	Powder
5101-125	Gamborg's B5 Complete Medium (Salts, Vitamins, Sucrose), 1L Pouch	Powder
5101-114	Gamborg's B5 Complete Medium (Salts, Vitamins, Sucrose), liquid, 500ml	Liquid
5101-225	Gamborg's B5 Complete Agar (Salts, Vitamins, Sucrose, Agar), 1L Pouch	Powder
5101-042	Gamborg's Vitamins Powder, 5g	Powder
5101-044	Gamborg's Vitamins (1000X) Liquid, 20ml	Liquid
5102-035	Chu's N6 Basal Salts, 10L Pouch	Powder
5102-014	Chu's N6 Basal Salts, 10X Liquid, 500ml	Liquid
5102-335	Chu's N6 Basal Medium (Salts, Vitamins), 10L Pouch	Powder
5102-125	Chu's N6 Complete Medium (Salts, Vitamins, Sucrose), 1L Pouch	Powder
5102-114	Chu's N6 Complete Medium (Salts, Vitamins, Sucrose), Liquid, 500ml	Liquid
5102-225	Chu's N6 Complete Agar (Salts, Vitamins, Sucrose, Agar), 1L PouchPowder	
5102-042	Chu's N6 Vitamins Powder, 5g	Powder
5102-044	Chu's N6 Vitamins (1000X) Liquid, 20ml	Liquid
Culture Dishes		
5110-033	Magenta Box GA7, Pack of 8	Pack
5110-053	Magenta Box GA7, 5 packs of 8	Pack
5110-043	Magenta Box Tray (7-Way)	Each
5111-033	Petri Dishes, Sterile Polystyrene, 100mm x 20mm, 20 plates	Sleeve
5111-053	Petri Dishes, Sterile Polystyrene, 100mm x 20mm, 25 sleeves of 20	Sleeve

Cat. #	Description & Size
Nucleic Acid Purification Products	
6045-050	FastRNA® Pro Green Kit (Plants and Animals), 50 preps
6540-400	FastDNA® Kit, 100 preps
6560-200	FastDNA® SPIN Kit for Soil, 50 preps
6001-100	FastPrep® FP100A Instrument, 100V
6001-120	FastPrep® FP120A Instrument, 120V
6001-220	FastPrep® FP220A Instrument, 220V
6025-050	FastRNA® Pro Blue Kit (Bacteria), 50 preps
6035-050	FastRNA® Pro Red Kit (Yeast and Fungi), 50 preps
2012-400	Floraclean™ Kit, 25 preps

9. Product Use Limitation & Warranty

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