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TECHNICAL INFORMATION CONTENTS

ORGANIZATION OF A TISSUE CULTURE LABORATORY

Any laboratory in which tissue culture techniques are performed, regardless of the specific purpose, must contain a number of basic facilities. These usually include the following:

- A general washing area
- A media preparation, sterilization, and storage area
- An aseptic transfer area
- Environmentally controlled incubators or culture rooms
- An observation/ data collection area.

Washing Area

The washing area should contain large sinks, draining boards, and racks, and have access to deionized/distilled water. Space for drying ovens or racks, automated dishwashers, acid baths, pipet washers and driers, and storage cabinets may be necessary in the washing area, depending on the work being performed.

Media Preparation Area

The media preparation area should have ample storage space for the chemicals, culture vessels and closures, and glassware required for media preparation and dispensing. Bench space for hot plates/ stirrers, pH meters, balances, water baths, and media-dispensing equipment should be available. Other necessary equipment may include air and vacuum sources, Bunsen burners with a gas source, refrigerators and freezers for storing stock solutions and chemicals, a microwave or convection oven, and an autoclave or domestic pressure cooker for sterilizing media, glassware, and instruments.

In preparing culture media, analytical grade chemicals should be used and good weighing habits practiced. To insure accuracy, an exact, step-by-step routine should be developed for media preparation. This routine should be contained in a complete media preparation checklist required to be completed by all media preparers, even for the simplest media.

The water used in preparing media should be highly purified though deionization and/or distillation. Tap water is not recommended because it may contain undesirable salts and dissolved gases, microorganisms (algae, fungi, bacteria), and particulate matter (silt, oils, organic matter, etc.). Water used for plant tissue culture should meet, at a minimum, the standards for type II reagent grade water, i.e., be free of pyrogens, gases, and organic matter and have an electrical conductivity less than 1.0 µmho/cm.

The most common and preferred method of purifying water to type II standards is a deionization treatment followed by one or two glass distillations. The deionization treatment removes most ionic impurities, and the distillation process removes large organic molecules, microorganisms, and pyrogens. Three other methods that will produce type II purity water are absorption filtration, which uses activated carbon to remove organic contaminants and free chlorine; membrane filtration, which removes particulate matter and most bacterial contamination; and reverse osmosis, which removes approximately 90% of the bacterial, organic, and particulate matter as well as about 90% of the ionized impurities.

Transfer Area

Under very clean and dry conditions, tissue culture techniques can be successfully performed on an open laboratory bench. However, it is advisable that a laminar flow hood or sterile transfer room be utilized for making transfers. Within the transfer area there should be a source of electricity, gas, compressed air, and vacuum.

The most desirable arrangement is a small dust-free room equipped with an overhead ultraviolet light and a positive-pressure ventilation unit. The ventilation should be equipped with a high-efficiency particulate air (HEPA) filter. A 0.3-µm HEPA filter of 99.97-99.99% efficiency works well. All surfaces in the room should be designed and constructed in such a manner that dust and microorganisms do not accumulate and the surfaces can be thoroughly cleaned and disinfected. A room of such design is particularly useful if large numbers of cultures are being manipulated or large pieces of equipment are being utilized.

Another type of transfer area is a laminar flow hood. Air is forced into the unit through a dust filter then passed through a HEPA filter. The air is then either directed downward (vertical flow unit) or outward (horizontal flow unit) over the working surface. The constant flow of microbe-free filtered air prevents non-filtered air and particulate matter in the room from settling on the working surface.

The simplest type of transfer area suitable for tissue culture work is an enclosed plastic box, commonly called a glove box. This type of culture hood is sterilized by an ultraviolet light and wiped down periodically with 70% alcohol when in use. This type of unit is used when relatively few transfers are performed.

Culture Room

All types of tissue cultures should be incubated under conditions of well-controlled temperature, humidity, air circulation, and light quality and duration. These environmental factors may influence the growth and differentiation process directly during culture or indirectly by affecting their response in subsequent generations. Protoplast cultures, low-density cell suspension cultures, and anther cultures are particularly sensitive to environmental condition.

Typically, the culture room for growth of plant tissue cultures should have a temperature between 15° and 30° C, with a temperature fluctuation of less than ±0.5° C; however, a wider range in temperature may be required for specific experiments. It is also recommended that the room have an alarm system to indicate when the temperature has reached preset high or low temperature limits, as well as a continuous temperature recorder to monitor temperature fluctuations. The temperature should be constant throughout the entire culture room (i.e., no hot or cold spots). The culture room should have enough fluorescent lighting to reach 10,000 lux; the lighting should be adjustable in terms of quantity and photoperiod duration. Both light and temperature should be programmable for a 24-hr period. The culture room should have fairly uniform forced-air ventilation, and a humidity range of 20-98% controllable to ±3%. Many incubators, large growth chambers, and walk-in environmental chambers meet these specifications.

BASIC LABORATORY EQUIPMENT

Many tissue culture techniques require similar basic laboratory equipment. The following items are commonly found in a laboratory for *in vitro* propagation of plant materials:

Items Typically Needed to Start a Plant Cell/ Tissue Culture Laboratory

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US$)	ITEM FUNCTION
1	Water Purification System; water should have a resistivity of at least 200,000 ohms/cm and a conductivity 5.0 micromhos/cm	NA		Purification of water for media preparation
1	Electronic Balance (0.01 g readability; 200 g minimum capacity)	B798	334.50	Measuring out biochemicals and media
1	pH meter (range 0-14 +/- 0.01; automatic temperature compensation 0-60^0 C; one or two point calibration)	P099	96.50	Measurement and adjustment of media pH
1	Hot Plate/ Stirrer (7” x 7” ceramic top; variable heating range from ambient to 400^0 C; variable stirring speed from 50-150 rpm; chemically resistant)	H926	334.50	Mixing & heating media and stock
1	Refrigerator/ freezer; capable of maintaining a refrigerator temperature of 2-6^0 C with a freezer temperature of approximately –20^0 C	NA		Storage of stock solutions, media, plant growth regulators, etc.
1	Laminar Flow Transfer Hood; incoming air should be HEPA filtered to remove 99.99% of particles larger that 0.3 µm; should meet or exceed the Class 100 Clean Standard 209D; should maintain a flow of 90 fpm +/- 20% at static pressures of 0.6-1.2”	NA		Provide a sterile atmosphere to transfer cultures
1	4 liter 70% Isopropyl alcohol (or several pint bottles purchased from a local pharmacy)	NA		Used to sterilize instruments and work areas
1 roll	Aluminum foil, heavy duty; (18” x 500 ft roll)	F035	31.50	Used to wrap instruments prior to sterilization, cover vessels

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US$)	ITEM FUNCTION
12	Beakers, 250 mL	B910	9.50	Mixing solutions
12	Beakers, 1000 mL	B931	16.50	Mixing solutions
6	Beakers, 2000 mL	B939	37.50	Mixing solutions and media
6	Beakers, 4000 mL	B960	53.50	Mixing solutions and media
4 ea	Bottle, Water; pre-labeled for use in dispensing water; 500 mL capacity	B987	7.50	Rinsing instruments, beakers, transplants from tissue culture
4 ea	Bottle, Isopropyl Alcohol; pre-labeled for use in dispensing isopropyl alcohol; 500 mL capacity	B974	7.50	Rinsing sterile hood work surfaces
1 case	Bottle, 500 mL; Type 1 borosilicate glass with volume graduations; supplied with 33-340 black polypropylene cap with rubber liner; non-sterile	B177	8.75	Storage of stock solutions, sterile distilled water, media
1 case	Bottle, 1000 mL; Type 1 borosilicate glass with volume graduations; supplied with 33-340 black polypropylene cap with rubber liner; non-sterile	B183	12.50	Storage of stock solutions, sterile distilled water, media
2	Brushes, flask or bottle	NA		Cleaning glassware
	Culture Vessels & Accessories:			
1 case	Culture tubes, 25 x 150 mm, borosilicate glass; 500 tubes/ case	C930	166.50	Starting cultures in Stage I
1 case	Culture tube racks; holds 40, 25 mm culture tubes; withstands temperatures up to 121^0 C	C908	68.75	Holding culture tubes
500	Closures, for 25 mm culture tubes, 500 each	C609	65.50	Sealing culture tubes
1 case	Culture vessel, *Phyto*Tech PC-120™; autoclavable; supplied with *Phyto*Cap (C070) as closure; 100/ case	C185	144.50	Culture vessel for maintaining plant cultures
1 case	*Phyto*Tech Culture Box™; presterilized (not autoclavable; 250/ case	C700	157.50	Culture vessel for maintaining plant cultures
1 case	*Phyto*Caps; autoclavable closure for baby food jars (fits both C904 and C900) & C185; clear polypropylene closure; 100/ case	C070	157.50	Closure for baby food culture vessels & PC-120 containers
1 case	Culture vessels; autoclavable culture vessel and lid made from clear polypropylene; round vessel measures; 250/ case	C209 C215 C221	94.50 101.50 159.50	Culture vessel for maintaining plant cultures
1 liter	Detergent	C284	15.00	Cleaning glassware
1 case	Culture dishes, disposable, sterile, 100 x 15 mm	D940	63.75	Sterile surface for cutting explants (for Stage I cultures)
1 gal	Chlorine bleach (sodium hypochlorite)	NA		Surface sterilize explants
6	Erlenmeyer flask, 250 mL	F979	35.50	Mixing media
6	Erlenmeyer flask, 500 mL	F980	35.50	Mixing media
1	Erlenmeyer flask, wide mouth, 1000 mL	F985	21.50	Mixing media
1	Erlenmeyer flask, wide mouth, 2000 mL	F986	30.50	Mixing media
1 dozen	Filtration system, vacuum; disposable, plastic, sterile system designed for filtration of fluids needed in tissue culture; polystyrene, screw-cap base for sterile storage; 200 mL; 47 mm diameter/ 0.22 µm pore size nylon membrane	NA		Sterilization of heat labile stock solutions

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US$)	ITEM FUNCTION
3	Forceps, dressing; 10” length, serrated, stainless steel	F952	13.50	Transferring tissue
3	Forceps, bayonet; stainless steel	F957	30.50	Transferring tissue
3	Forceps; stainless steel, very fine point, 5” length	F090	22.25	Transferring tissue
1	Graduated cylinder, glass or plastic, 10 mL	C962	5.50	Preparing stock solutions
1	Graduated cylinder, glass or plastic, 100 mL	C964	8.50	Preparing stock solutions
1	Graduated cylinder, glass or plastic, 1000 mL	C968	23.50	Preparing stock solutions
1 pkg	Lab markers, assorted colors (10)	NA		Labeling cultures
1 roll	Parafilm (4” x 250 ft)	S911	41.50	Wrapping culture closures
100 ea	Pipets, 1 mL, graduated, sterile, disposable, individually wrapped	P990	7.50	Measuring out stock solutions
100 ea	Pipets, 5 mL, graduated, sterile, disposable, individually wrapped	P993	11.50	Measuring out stock solutions
100 ea	Pipets, 10 mL, graduated, sterile, disposable, individually wrapped	P994	10.50	Measuring out stock solutions
100 ea	Pipets, 25 mL, graduated, sterile, disposable, individually wrapped	P995	24.50	Measuring out stock solutions
1	Pipet pump, electric	P071, P072	294.50	For safely measuring liquids
1 pkg	Gloves, hot or autoclave; provides protection up to 350^0 F	G089 G092	30.50 23.50	Safely removing hot items from autoclave
2 ea	Scalpel handle; No 3: 5” length; stainless steel	S963	18.50	Cutting explants
2 ea	Scalpel handle; No 3L: 8” length; stainless steel	S973	21.50	Cutting explants
1 box	Scalpel blades; No 10; stainless steel; individually wrapped; sterile; 100/ box	S970	67.50	Cutting explants
1 ea	Scoop, large plastic 5-3/4” x 9” bowl	NA		Measuring large volumes of biochemicals
1	Spatula, 6” V-shaped, plastic	S830	2.50	Measuring small to medium amounts of biochemicals
2	Spatula/ Spoon, double-ended, stainless steel	S978	5.50	Measuring small to medium amounts of biochemicals
2	Spatula, double-ended, stainless steel	S977	4.50	Measuring small to medium amounts of biochemicals; rounded ends, 2” end length
1 ea	Sterilizer, pressure cooker; operates between 116-126^0 C; 10-20 psi; aluminum sterilizer has a 30 x 32.2 cm chamber; is supplied with chamber, lid with pressure gauge, immersion heater and safety valve, electric (for small operations)	NA		Sterilizing media and instruments
1 ea	Sterilizer, autoclave; operates at 121^0 C with dial for fast or slow exhaust; 0-60 minute timer; stainless unit with 66 x 41 cm chamber (for large operations)	NA		Sterilizing media and instruments
1 ea	Sterilizer, dry heat with glass beads 115-120 V	S708	415.00	Sterilizes instruments in hood between transfers

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US$)	ITEM FUNCTION
1 pkg	Stir bars, magnetic; Teflon covered	B010, B011, B012, B013, B015, B017	Varies	Used for mixing stock solutions and in media preparation
1 ea	Stir bar retriever; contains a magnet sealed in polyethylene; 18" length	B016	9.50	Retrieving stir bars from mixing vessel
1 roll	Tape, autoclave indicator; impregnated to show the word "Autoclaved" after 15 minutes of exposure at 121^0 C	T998	4.50	Identifying autoclaved media
1 roll	Tape, label; all purpose, self adhesive tape can be written on with pen or permanent marker	T690, T695 F696, T697 T699, T706	4.50	Labeling cultures, storage bottles, media vessels, etc.
2 ea	Thermometer; digital, -20-200^0 C temperature range	T786	31.26	Measuring temperature of liquids and culture room
1	Timer; electronic, countdown timer alarm, stopwatch feature	NA		Timing sterilization and general lab use
1 case	Towels; commercial, single fold	NA		Can be sterilized to provide sterile work surface for cutting explants, general lab use
1 each	Tool rests; horizontal (I556), vertical (I623), or wire (I718)	I556, I623 I718	23.50	Holds instruments after sterilization
500 ea	Weigh boats, small, plastic; can be utilized for liquid or solid samples; 1-5/8" x 1-5/8" x 5/16"	W879	31.50	Measuring chemicals
500 ea	Weigh boats, medium, plastic; can be utilized for liquid or solid samples; 3-5/16" x 3-5/16" x 1"	W880	26.50	Measuring chemicals
500 ea	Weigh boats, large, plastic; can be utilized for liquid or solid samples; 5-1/2" x 5-1/2" x 1"	W881	87.50	Measuring chemicals

The glassware used in tissue culture can generally be found in most laboratories. The glassware, particularly the culture vessels, should be made of Pyrex or borosilicate glass. Due to the increasing expense of this type of glass, many laboratories are successfully converting to soda glass, which may be significantly cheaper. Wide-neck Erlenmeyer flasks (50-, 125-, 250-mL capacity) are commonly used as culture vessels; large volume Erlenmeyer flasks are required for media preparation. Test tubes, Petri dishes, mason jars, baby food jars, and other glassware can also be adapted to tissue culture. Since new glass may release substances that affect the composition of the medium, it is recommended that all new glassware be filled with water, autoclaved twice with detergent, and rinsed between autoclaving before being used for tissue culture. Other glassware commonly required in a tissue culture facility includes beakers, volumetric flasks, pipets, and graduated cylinders.

BASIC LABORATORY PROCEDURES

The majority of laboratory operations utilized in the *in vitro* propagation of plants can be easily learned. One needs to concentrate mainly on accuracy, cleanliness, and strict adherence to details when performing *in vitro* techniques.

Weighing

The preparation of media requires careful weighing of all components. Even if a commercially prepared medium is used, care must be taken in preparing it and any stock solutions that are required.
Because of the diversity of laboratory balances, it is impossible to review the details of their operation. The manufacturer's instructions should be consulted before using any balance. The types of balances most often encountered in the laboratory include the top-loading single-pan balance, triple-beam balance, double-pan torsion balance, analytical single-pan balance, and top-loading electronic balance. The last type has become quite popular in recent years due to its accuracy, ease of use, and durability. With certain models of top-loading electronic balances, milligram accuracy is possible. Such accuracy previously required the use of analytical balances.

Several common precautions must be observed to obtain accurate weights. First, the balance should be located on a hard, stable, level surface which is free of vibrations and excessive air drafts. The balance or weigh area should always be kept clean. Most importantly, the balance should *never* be overloaded (see manufacturer's specification). It is advisable to use a lightweight weighing container or paper rather than placing the material to be weighed directly on the pan surface.

Measuring Liquids

Calibrated glassware (e.g., beakers, flasks, and pipets) are required for the preparation of culture media. Graduated cylinders of 10-, 25-, 100-, and 1000 mL capacities are used for many measuring operations, but volumetric flasks and pipets are required for more precise measurements. Measurement of solutions with pipets or graduated cylinders is only accurate when the bottom of the curved air-liquid interface is aligned with the measuring mark.

Pipets should be filled with a hand-operated device, called a pipettor, which eliminated the hazards of pipetting by mouth. Never pipet by mouth! Three types of pipettors are commonly used. The first is a bulb-type pipettor, which is controlled by a series of valves. The second type of pipettor is operated simply by rotating a small wheel on the side of the handle. Rotating the wheel upward creates a suction bringing the liquid into the pipet; rotating the wheel in the opposite direction releases the liquid. A third type of pipettor utilizes an electric air pump. Liquid is drawn into the pipet and released by pressing the appropriate buttons.

Cleaning Glassware

The conventional method of washing glassware involves soaking glass in a chromic acid-sulfuric acid bath followed by tap water rinses, distilled water rinses, and finally double-distilled water rinses. Due to the corrosive nature of chromic acid, the use of this procedure has been eliminated except for highly contaminated or soiled glassware. Adequate cleaning of most glassware for tissue culture purposes can be achieved by washing in hot water (70° C+) with commercial detergents, rinsing with hot tap water (70° C+), and finally rinsing with distilled and double-distilled water. However, highly contaminated glassware should be cleaned in a chromic acid-sulfuric acid bath or by some other proven method such as (1) ultrasonic cleaning, (2) washing with sodium pyrophosphate, or (3) boiling in metaphosphate (e.g., Alconox®), rinsing then boiling in a dilute hydrochloric acid solution, and then finally rinsing in distilled water. Cleaned glassware should be inspected, air dried (or preferably at 150° C in a drying oven), capped with aluminum foil, and stored in a closed cabinet.

The following general procedure is recommended for cleaning glassware that contains media and cultures after all data have been collected:

1. Autoclave all glassware with media and cultures still in it. This kills any contaminating microorganisms that may be present.
2. After the autoclaved media has cooled, but while it is still in a liquid state, pour it into bio-hazard plastic bags or thick plastic bags, seal, then discard.
3. Wash all glassware in hot soapy water using a suitable bottle brush to clean the internal parts of the glassware. Any glassware that is stained should be soaked in a concentrated sulfuric acid-potassium dichromate acid bath for 4 hr, and then thoroughly rinsed before washing it with soapy water.
4. All glassware should be rinsed three times in tap water, three times in deionized/ distilled water, dried, and stored in a clean place.
5. Wash all instruments and new glassware in a similar manner.

Sterilization

The most critical parts of *in vitro* techniques are sterilizing plant materials and media and maintaining aseptic conditions once they have been achieved. Bacteria and fungi are the two most common contaminants observed in cell cultures. Fungal spores are lightweight and present throughout our environment. When fungal spores come into contact with the culture media used in tissue culture, conditions are optimal for germination of the spores and subsequent contamination of the culture.

Sterilizing Culture Rooms and Transfer Hoods

Large transfer rooms are best sterilized by exposure to ultraviolet (UV) light. Sterilization time varies according to the size of the room and should only be done when there are no experiments in progress. Ultraviolet radiation is harmful to the eyes. Transfer rooms can also be sterilized by washing them 1-2 times a month with a commercial brand of disinfectant. Smaller transfer rooms and hoods also can be sterilized with UV lights or by treatment with disinfectants. Laminar flow hoods are easily sterilized by turning on the hood and wiping down all surfaces with 70% alcohol 15 min before initiating any operation under the hood. Culture rooms should be initially cleaned with a disinfecting detergent and then carefully wiped down with a 2% sodium hypochlorite solution or 70% alcohol. All floors and walls should be washed gently on a weekly basis with a similar solution; extreme care must be used to avoid stirring up any contamination that has settled. Commercial disinfectants such as Lysol® and Roccal® diluted at manufacturer's recommended rates can be used to disinfect work surfaces and culture rooms.

Sterilizing glassware and Instruments

Metal instruments are best sterilized using a glass bead sterilizer, Product Number S708. These sterilizers heat to approximately 230-250° C and will destroy bacterial and fungal spores that may be found on your instruments. The instruments simply need to be inserted into the heated glass beads for a period of 10 to 60 sec. The instruments should then be placed on a sterile rack under the hood to cool until needed. Metal instruments, glassware, aluminum foil, etc., can also be sterilized by exposure to hot dry air (250° C) for 2-4 hr in a hot-air oven. All items should be sealed before sterilization in aluminum foil or heat-resistant bags. Autoclaving is not advisable for metal instruments (esp. low-grade stainless and carbon steel) because they may rust and become blunt under these conditions.

After an instrument has been used, it can again be dipped in ethyl alcohol, re-flamed, and then reused. This technique is called flame sterilization. Safety is a major concern when using ethyl alcohol. Alcohol is flammable and if spilled near a flame will cause a flash fire. This problem is compounded in laminar flow hoods due to the strong air currents blown towards the worker. Fires commonly start when a flamed instrument is thrown back into the alcohol beaker. In case of fire do not panic. Limiting the supply of oxygen by covering the container of alcohol (e.g., glass Petri plate) can easily put out a fire.

Autoclaving is a method of sterilizing with steam under pressure. Cotton plugs, gauze, lab ware, plastic caps, glassware, filters, pipets, water, and nutrient media can all be sterilized by autoclaving. Microbes are killed by exposure to the super-heated steam of an autoclave for 10-15 minutes. All objects should be sterilized at 121° C and 15 psi for 15-20 min.

Sterilizing Nutrient Media

Two methods (autoclaving and membrane filtration under positive pressure) are commonly used to sterilize culture media. Culture media, distilled water, and other heat-stable mixtures can be autoclaved in glass containers that are sealed with cotton plugs, aluminum foil, or plastic closures. However, solutions that contain heat-labile components must be filter-sterilized.

Generally, nutrient media are autoclaved at 15 psi and 121° C. For small volumes of liquids (100 mL or less), the time required for autoclaving is 15-20 min, but for larger quantities (2-4 liter), 30-40 min or longer is required. The pressure should not exceed 20 psi, as higher pressures may lead to the decomposition of carbohydrates and other thermo-labile components of a medium. Since certain proteins, vitamins, amino acids, plant extracts, hormones, and carbohydrates are thermo-labile and may decompose during autoclaving, filter sterilization may be required. There are many brands of filters available today. These are typically purchased pre-sterilized. The porosity of the filter membrane should be no larger than 0.2 micrometers (µm). Empty glassware that is to hold filter-sterilized media must be sterilized in an autoclave before filter sterilization.

Nutrient media that contain thermo-labile components can be prepared in several steps. That is, a solution of the heat-stable components is sterilized in the usual way by autoclaving, and then cooled to 50°-60° C under sterile conditions. In a separate operation, solutions of the thermo-labile components are filter-sterilized. The sterilized solutions are then combined under aseptic conditions to give the complete media.

Surface-Sterilizing (Disinfesting) Plant Material

Obtaining sterile plant material is difficult, and despite any precautions taken, 95% of cultures will end up contaminated if the explant is not disinfested in some manner. Because living materials cannot be exposed to extreme heat and retain their biological capabilities, plant organs and tissues are surface-sterilized by treatment with a disinfecting solution. These solutions used to disinfest explants must preserve the plant tissue but at the same time destroy any fungal or bacterial contaminants.

Once explants have been obtained, they should be washed in a mild soapy detergent before treatment with a sterilizing solution. Some herbaceous plant materials (e.g., African violet leaves) may not require this step, but woody material, tubers, etc., must be washed thoroughly. After the tissue is washed, it should be rinsed under running tap water for 10-30 min and then be submerged into the disinfectant under sterile conditions. All surfaces of the explant must be in contact with the sterilant. After the allotted time for sterilization, the sterilant should be decanted and the explants washed at least three times in sterile distilled water. For materials that are difficult to disinfest, it may be necessary to repeat the treatment 24-48 hr before culturing the final explants. This allows previously viable microbes time to develop to a stage at which they are vulnerable to the sterilant.

Sterile Culture Techniques

Successful control of contamination depends largely upon the operator's techniques in aseptic culture. You should always be aware of potential sources of contamination such as dust, hair, hands, and clothes. Obviously, your hands should be washed, sleeves rolled up, long hair tied back, etc. Washing your hands with 70% alcohol is an easy precautionary measure that can be taken. Talking or sneezing while culture material is exposed also can lead to contamination. When transferring plant parts from one container to another, do not touch the inside edges of either vessel. By observing where contamination arises in a culture vessel, you may be able to determine the source of the contaminant.

In plant tissue culture, small pieces of plant tissue are placed on or in a medium rich in nutrients and sugar. If bacteria or fungi come in contact with the plant tissue or the medium, the culture becomes contaminated. The contaminants (bacteria and fungi) will use nutrients from the medium and quickly multiply and overwhelm the culture. Pathogenic contaminants will also destroy the plant tissue. Our aim is to surface sterilize the plant tissue and put it on a sterile growth medium without any bacteria or fungi getting on the plant or medium. This is not easy because bacteria and fungal spores are in the air, on us, in us, and under us! When you see sunlight shining in a window you can, from certain angles, see dust particles in the air. There are hundreds of bacteria attached to each dust particle. A horizontal laminar flow unit is designed to remove the particles from the air. Room air is pulled into the top of the unit and pushed through a HEPA (High Efficency Particulate Air) filter with a uniform velocity of 90 ft/min across the work surface. The air is filtered by the HEPA filter so nothing larger than 0.3 µm (micrometer) can pass through. This renders the air sterile. The flow of air from the unit prevents fungal spores or bacteria from entering. All items going inside the unit should be sterile or sprayed with alcohol. They will remain sterile unless you contaminate them.

A transfer cabinet provides an enclosed environment that is not sterile but can be sterilized. A cardboard box lined with aluminum foil or plastic, or a well-cleaned aquarium, provides a shield to reduce contamination. A box that is 20-24 inches wide, 20-24 inches high, and 12-16 inches deep provides a good work area. Working inside any of these does not guarantee success.

When working in a group, the following precautions are necessary for all work areas.

1. The room should be swept and if possible, mopped.
2. Each work surface should be washed with 10% chlorine bleach, Lysol®, or other disinfectant solution.
3. Doors and windows should be closed.
4. Air conditioners and fans should be turned off.
5. If possible, each person should have a work space that can be properly treated against contamination (e.g., the box or aquarium mentioned earlier, or a piece of poster paper lying on the table to indicate the student's sterile workspace).
6. Have spray bottles filled with 70% ethanol or isopropanol (never methanol) placed so each person has access to one bottle. Spray everything going into the sterile area.
7. Have a central supply area so all necessary items can be picked up and taken to the workspace as needed. Items can be returned to the central supply area after being used.
8. Sterile instruments will be needed for each person. One way to accomplish this is to have a ½-pint jar of 70% ethanol for scalpels and short forceps. When tissue has to be positioned in a vessel, long 10-inch forceps are needed. The long forceps need to be placed deep enough in alcohol so that any part of the forceps that might come into contact with the vessel is sterilized. A 100 mL graduated cylinder can be used to hold the alcohol and long forceps. A ½-pint jar of sterile water is needed for dipping the instruments to remove the residual alcohol that might dry out plant tissues.

9. A sterile work surface is needed on which to place the sterile tissue to trim it. A sterile Petri dish is a convenient surface upon which to work. Glass Petri dishes can be autoclaved and reused. Pre-sterilized plastic dishes are used and discarded. Spray the bag of dishes with 70% alcohol before you open it and place the desired number of unopened dishes at each station. Each dish has two sterile surfaces-the inside top and inside bottom.
10. Long hair should be tied back or covered. Hands should be washed, not scrubbed (scrubbing dries hands and creates flakes of skin that have bacteria) and sprayed with 70% ethyl or isopropyl alcohol or coated with isopropyl alcohol gel. Gloves and masks provide extra protection. Do not talk while performing sterile operations. Do not lean over the work area. Keep your back against the backrest of your chair. Try to work with your arms straight; this position may feel awkward, but it will reduce contamination. Do not pass non-sterile items over sterile areas or items. Reach around rather than over. Make your movements smooth and graceful so that you do not disturb the air more than is necessary. Work quickly though, the longer it takes to manipulate the tissues the greater the chance of contamination. Have only the necessary items in the sterile work area. Remove items that are no longer needed as quickly as possible. Think about each step before beginning so that you understand what you are about to do.

Store cultures in a well-lit area (not in direct sunlight), and do not allow the temperature to exceed 80^0 F where the cultures are stored. If cultures are to be placed under lights, use only fluorescent lighting. The preferred schedule is 16 hours of light and 8 hours of dark. Check the temperature prior to placing the cultures under the lights because temperature will build even under fluorescent lights.

Check cultures every 3-5 days for contamination. Slimy areas mean bacterial contamination while fuzzy areas are due to fungal contamination. *Do not open containers that are contaminated.* The contaminants could be harmful. The only safe way to dispose of these is to autoclave (or pressure-cook) them for 15 minutes at 15 psi. Contaminated plastic dishes can be placed inside a large can or autoclavable bag to be sterilized before discarding.

Determining pH

The pH of a solution is a measure of the concentration of hydrogen ions in the solution. The pH scale extends from very acid (0) to very alkaline (14) with 7 being the "neutral" point. The pH of most culture media is adjusted to 5.7 ± 0.1 before autoclaving. The pH can influence the solubility and availability of ions in nutrient media, the ability of agar to gel, and the subsequent growth of cells. Thus accurate determination and control of media pH are necessary. Generally, pH is determined with a pH meter.

LABORATORY SAFETY AND DAILY MAINTENANCE OPERATIONS

Observing commonsense safety practices can significantly reduce the possibility of accidents or injuries occurring in a laboratory. For your safety and that of others, observe the following:

- Always wear shoes and a laboratory jacket in the laboratory.
- Be extremely careful handling alcohol around open flames. It is flammable!
- Never pipet by mouth.
- Handle hydrochloric acid, sulfuric acid, sodium hydroxide, and other strong acids and alkalis (bases) with extreme caution. They are very corrosive!
- Wash and bandage all cuts immediately.
- Before opening an autoclave, be sure the pressure is reduced to zero and the temperature is below 100° C.

In addition to safety concerns, cleanliness and proper care of equipment are vital to the efficient operation of a tissue culture laboratory. The following tasks should be performed routinely before the laboratory is closed at night:

1. Mop floors in the lab and culture room with an approved disinfectant.
2. Turn off hood, unless used continuously to reduce particulates in the air.
3. Fill distilled water tanks and turn off stills.
4. Clean off benches *completely* and put away chemicals, instruments, glassware, etc.
5. Put microscopes on lowest magnification; turn off, and cover them.
6. Shut off all water outlets.
7. Wash and dry all dirty glassware (tubes, pipets, flasks, etc.).
8. Put away all clean, dry glassware, racks, etc.
9. Turn off all electrical equipment that is not in use overnight (e.g., stirrers, pH meters, balances).
10. Put all chemicals, reagents, and solutions in their proper storage areas.

TISSUE CULTURE MEDIA COMPOSITION

Media Components

One of the most important factors governing the growth and morphogenesis of plant tissues in culture is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants.

Plant tissue and cell culture media are generally made up of some or all of the following components: macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, sugar(s), other undefined organic supplements, solidifying agents or support systems, and growth regulators. Several media formulations are commonly used for the majority of all cell and tissue culture work. These media formulations include those described by White (1963), Murashige and Skoog (1962), Gamborg, *et al.* (1968), Schenk and Hildebrandt (1972), Nitsch and Nitsch (1969), and Lloyd and McCown (1981). Murashige and Skoog (MS) medium, Schenk and Hildebrandt (SH) medium, and Gamborg's B-5 medium are all high in macronutrients, while the other media formulations contain considerably less of the macronutrients.

Macronutrients

The macronutrients provide the six major elements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) required for plant cell or tissue growth. The optimum concentration of each nutrient for achieving maximum growth rates varies considerably among species.

Culture media should contain at least 25-60 mM inorganic nitrogen for adequate plant cell growth. Plant cells may grow on nitrates alone. However, considerably better results are obtained with most plant species when the medium contains both a nitrate and ammonium nitrogen source. Certain species require ammonium or another source of reduced nitrogen for cell growth to occur. Nitrates are usually supplied in the range of 10-40 mM; typical ammonium concentrations range between 2 and 20 mM. However, ammonium concentrations in excess of 8 mM may be deleterious to cell growth of certain species. Cells can grow on a culture medium containing ammonium as the sole nitrogen source if one or more of the TCA cycle acids (e.g., citrate, succinate, or malate) are also included in the culture medium at concentrations of approximately 10 mM. When nitrate and ammonium sources of nitrogen are utilized together in the culture medium, the ammonium ions typically will be utilized more rapidly and before the nitrate ions.

Potassium (K) is required for cell growth of plant species. Most media contain K in the nitrate, sulfate, or chloride form, at concentrations of 20-30 mM. The optimum concentrations of phosphorus (P), magnesium (Mg), sulfur (S), and calcium (Ca) range from 1-3 mM when all other requirements for cell growth are satisfied. Higher concentrations of these nutrients may be required if deficiencies in other nutrients exist.

Micronutrients

The essential micronutrients for plant cell and tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo). Chelated forms of iron and zinc are commonly used in preparing culture media. Iron may be the most critical of all the micronutrients. Iron citrate and tartrate may be used in culture media, but these compounds are difficult to dissolve and frequently precipitate after media are prepared. Murashige and Skoog used an ethylene diaminetetraacetic acid (EDTA)-iron chelate to circumvent this problem.

Cobalt (Co) and iodine (I) may also be added to certain media, but strict cell growth requirements for these elements have not been established. Sodium (Na) and chlorine (Cl) are also used in some media but are not essential for cell growth. Copper and cobalt are normally added to culture media at concentrations of 0.1 µM, Fe and Mo at 1 µM, I at 5 µM, Zn at 5-30 µM, Mn at 20-90 µM, and B at 25-100 µM.

Carbon and Energy Source

The preferred carbohydrate in plant cell culture media is sucrose. Glucose and fructose may be substituted in some cases, glucose being as effective as sucrose and fructose being somewhat less effective. Other carbohydrates that have been tested include lactose, galactose, rafinose, maltose, and starch. Sucrose concentrations of culture media normally range between 2 and 3 percent. Use of autoclaved fructose can be detrimental to cell growth.

Carbohydrates must be supplied to the culture medium because few plant cell lines have been isolated that are fully autotropic, i.e., capable of supplying their own carbohydrate needs by CO_2 assimilation during photosynthesis.

Vitamins

Normal plants synthesize the vitamins required for their growth and development. Vitamins are required by plants as catalysts in various metabolic processes. When plant cells and tissues are grown *in vitro*, some vitamins may become limiting factors for cell growth. The vitamins most frequently used in cell and tissue culture media include thiamine (B_1), nicotinic acid, pyridoxine (B_6), and *myo*-inositol. Thiamine is the one vitamin that is basically required by all cells for growth. Thiamine is normally used at concentrations ranging from 0.1 to 10.0 mg/L. Nicotinic acid and pyridoxine are often added to culture media but are not essential for cell growth in many species. Nicotinic acid is normally used at concentrations of 0.1-5.0 mg/L; pyridoxine is used at 0.1-10.0 mg/L.

Myo-inositol is commonly included in many vitamin stock solutions. Although it is a carbohydrate not a vitamin, it has been shown to stimulate growth in certain cell cultures. Its presence in the culture medium is not essential, but in small quantities *myo*-inositol stimulates cell growth in most species. *Myo*-inositol is generally used in plant cell and tissue culture media at concentrations of 50-5000 mg/L.

Other vitamins such as biotin, folic acid, ascorbic acid, pantothenic acid, vitamin E (tocopherol), riboflavin, and ρ-aminobenzoic acid have been included in some cell culture media. The requirement for these vitamins by plant cell cultures is generally negligible, and they are not considered growth-limiting factors. These vitamins are generally added to the culture medium only when the concentration of thiamine is below the desired level or when it is desirable to grow cells at very low population densities.

Amino Acids or Other Nitrogen Supplements

Although cultured cells are normally capable of synthesizing all of the required amino acids, the addition of certain amino acids or amino acid mixtures may be used to further stimulate cell growth. The use of amino acids is particularly important for establishing cell cultures and protoplast cultures. Amino acids provide plant cells with an immediately available source of nitrogen, which generally can be taken up by the cells more rapidly than inorganic nitrogen.

The most common sources of organic nitrogen used in culture media are amino acid mixtures (e.g., casein hydrolysate), L-glutamine, L-asparagine, and adenine. Casein hydrolysate is generally used at concentrations between 0.05% and 0.1%. When amino acids are added alone, care must be taken as they can be inhibitory to cell growth. Examples of amino acids included in culture media to enhance cell growth are glycine at 2 mg/L, L-glutamine up to 8 mM, L-asparagine at 100 mg/L, L-arginine and L-cysteine at 10 mg/L, and L-tyrosine at 100 mg/L. L-Tyrosine has been used to stimulate morphogenesis in cell cultures but should only be used in an agar medium. Supplementation of the culture medium with adenine sulfate can stimulate cell growth and greatly enhance shoot formation.

Undefined Organic Supplements

Addition of a wide variety of organic extracts to culture media often results in favorable tissue responses. Supplements that have been tested include protein hydrolysates, coconut water, yeast extracts, malt extracts, ground banana, orange juice, and tomato juice. However, undefined organic supplements should only be used as a last resort. Only coconut water, ground banana, and protein hydrolysates are used to any extent today. Protein (casein) hydrolysates are generally added to culture media at a concentration of 0.05-0.1%, while coconut water is commonly used at 5-20% (v/v).

The addition of activated charcoal (AC) to culture media may have a beneficial effect. The effect of AC is generally attributed to one of three factors: absorption of inhibitory compounds, absorption of growth regulators from the culture medium, or darkening of the medium. The inhibition of growth in the presence of AC is generally attributed to the absorption of phytohormones (growth regulators) to AC. α-Naphthaleneacetic acid (NAA), kinetin, 6-benzylaminopurine (BA), indole-3-acetic acid (IAA), and 6-(γ-γ-dimethylallylamino)purine (2iP) all bind to AC, with the latter two growth regulators binding quite rapidly. The stimulation of cell growth by AC is generally attributed to its ability to bind to toxic phenolic compounds produced during culture. Activated charcoal is generally acid-washed prior to addition to the culture medium at a concentration of 0.5% to 3.0%.

Solidifying Agents or Support Systems

Agar is the most commonly used gelling agent for preparing semisolid and solid plant tissue culture media. Agar has several advantages over other gelling agents. First, when agar is mixed with water, it forms a gel that melts at approximately 60°-100° C and solidifies at approximately 45° C; thus, agar gels are stable at all typical incubation temperatures. Additionally, agar gels do not react with media constituents and are not digested by plant enzymes. The firmness of an agar gel is controlled by the concentration

and brand of agar used in the culture medium and the pH of the medium. The agar concentrations commonly used in plant cell culture media range between 0.5% and 1.0%; these concentrations give a firm gel at the pH levels typical of plant cell culture media.

Another gelling agent commonly used for commercial as well as research purposes is Gellan Gum. This is a product of bacterial fermentation and should be used at 1.25-2.5 g/L, resulting in a clear gel which aids in detecting contamination.

Alternative methods of support have included use of perforated cellophane, filter paper bridges, filter paper wicks, polyurethane foam, and polyester fleece. Whether explants grow best on agar or on other supporting agents varies from one species of plant to the next.

Growth Regulators

Four broad classes of growth regulators are important in plant tissue culture: auxins, cytokinins, gibberellins, and abscisic acid. Miller and Skoog (1953) were the first to report that the ratio of auxin to cytokinin determined the type and extent of organogenesis in plant cell cultures. Both an auxin and cytokinin are usually added to culture media in order to obtain morphogenesis, although the ratio of hormones required for root and shoot induction is not universally the same. Considerable variability exists among genera, species, and even cultivars in the type and amount of auxin and cytokinin required for induction of morphogenesis.

The auxins commonly used in plant tissue culture media are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), and α-naphthaleneacetic acid (NAA). The only naturally occurring auxin found in plant tissues is IAA. Other synthetic auxins that have been used in plant cell culture include 4-chlorophenoxyacetic acid or ρ-chlorophenoxyacetic acid (4-CPA, PCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and 4-amino-3,5,6-trichloropicolinic acid (picloram).

The various auxins differ in their physiological activity and in the extent to which they move through tissue, are bound to the cells, or are metabolized. Naturally occurring IAA has been shown to have less physiological activity than synthetic auxins. Based on stem curvature assays, 2,4-D has eight to twelve times the activity, 2,4,5-T has four times the activity, PCPA and picloram have two to four times the activity, and NAA has two times the activity of IAA. Although 2,4-D, 2,4,5-T, PCPA, and picloram are often used to induce rapid cell proliferation, exposure to high levels or prolonged exposure to these auxins, particularly 2,4-D, results in suppressed morphogenetic activity. Auxins are generally included in a culture medium to stimulate callus production and cell growth, to initiate roots and to a lesser degree shoots, to induce somatic embryogenesis, and stimulate growth from shoot apices and shoot tip cultures.

The cytokinins commonly used in the culture media include 6-benzylaminopurine or 6-benzylaminopurine (BA), 6-(γ-γ-dimeth ylallylamino)purine (2iP), kinetin, and zeatin. Zeatin and 2iP are considered to be naturally occurring cytokinins, while BA and kinetin are synthetically derived cytokinins. Adenine, another naturally occurring compound, has a base structure similar to that of the cytokinins and has shown cytokinin-like activity in some cases. Many plant tissues have an absolute requirement for a specific cytokinin for morphogenesis to occur, whereas some tissues are considered to be cytokinin independent, i.e., no cytokinin or specific cytokinin is required for organogenesis.

The cytokinins are generally added to a culture medium to stimulate cell division, to induce shoot formation and axillary shoot proliferation, and to inhibit root formation.

The type of morphogenesis that occurs in a plant tissue culture largely depends upon the ratio and concentrations of auxin and cytokinin present in the medium. Root initiation of plantlets, embryogenesis, and callus initiation all generally occur when the ratio of auxin to cytokinin is high, whereas adventitious and axillary shoot proliferation occurs when the ratio is low. The concentrations of auxins and cytokinins are equally as important as their ratio.

Gibberellins (GA_3) and abscisic acid (ABA) are two other growth regulators occasionally used in culture media. Plant tissue cultures can usually be induced to grow without either GA_3 or ABA, although, certain species may require these hormones for enhanced growth. Generally, GA_3 is added to culture media to promote the growth of low-density cell cultures, to enhance callus growth, and to elongate dwarfed or stunted plantlets. Abscisic acid is generally added to culture media to either inhibit or stimulate callus growth (depending upon the species), to enhance shoot or bud proliferation, and to inhibit latter stages of embryo development.

STOCK SOLUTIONS

The use of stock solutions reduces the number of repetitive operations involved in media preparation and, hence, the chance of human or experimental error. Moreover, direct weighing of media components (e.g., micronutrients and hormones) that are required only in milligram or microgram quantities in the final formulation cannot be performed with sufficient accuracy for tissue culture work. For these components, preparation of concentrated stock solutions and subsequent dilution into the final media is standard procedure. In addition, concentrated solutions of some materials are more stable and can be stored for longer periods than more dilute solutions.

To prepare a stock solution, weigh out the required amount of the compound and place it in a clean flask. It is common practice to make a stock solution 10x, 100x, or 1000x depending upon the solubility of the compound. Once the chemical is in the flask, dissolve it in a small amount of water, ethyl alcohol, 1 N NaOH, or 1 N HCl. Next, slowly add double-distilled water to the flask, while agitating. Continue this until the proper volume is reached. Label the flask with the name of the solution, preparation and expiration dates, and the name of the person who prepared the solution. Certain items, e.g., IAA, must be prepared and stored in amber bottles to prevent photodecomposition. The volumes of stock solutions prepared at various concentrations that must be used to achieve various final concentrations are presented in tabular form in the Plant Growth Regulator Section.

Macronutrients

Stock solutions of macronutrients can be prepared at 10 times the concentration of the final medium. A separate stock solution for calcium salts may be required to prevent precipitation. Stock solution of macronutrients can be stored safely for several weeks in a refrigerator at 2°-6° C.

Micronutrients

Micronutrient stock solutions are generally made up at 100 times their final strength. It is recommended that micronutrient stocks be stored in either a refrigerator or freezer until needed. Micronutrient stock solutions could be stored in a refrigerator for up to 1 year without appreciable deterioration. Iron stock solutions should be prepared and stored separately from other micronutrients in an amber storage bottle. Formulations for preparing stock solutions of iron are presented later.

Vitamins

Vitamins are prepared as 100x or 1000x stock solutions and stored in a freezer (-20° C) until used. Vitamin stock solutions should be made up each time media is prepared if a refrigerator or freezer is not available. Vitamin stock solutions can be stored safely in a refrigerator for 2-3 months but should be discarded after that time**.**

Growth Regulators

The auxins NAA and 2,4-D are considered to be stable and can be stored at 4° C for several months; IAA should be stored at -20° C. Auxin stock solutions are generally prepared at 100x to 1000x the final desired concentrations. Solution of NAA and 2,4-D can be stored for several months in a refrigerator or approximate one year at -20° C. Generally IAA and 2,4-D are dissolved in a small volume of 95% ethyl alcohol or KOH and then brought to volume with double-distilled water; NAA can be dissolved in a small amount of 1 N NaOH or KOH which also can be used to dissolve 2,4-D and IAA.

The cytokinins are considered to be stable and can be stored at -20° C. Cytokinin stock solutions are generally prepared at 100x to 1000x concentrations. Many of the cytokinins are difficult to dissolve, and a few drops/ mL of either 1 N HCL, 1 N NaOH/ KOH, or DMSO are required to bring them into solution.

Storage of Stock Solutions

Storage conditions for most stock solutions have already been pointed out; however, some additional points can be made. For convenience, many labs prepare stock solutions and then divide them into aliquots sufficient to prepare from 1 to 10 liters of medium; these aliquots are stored in small vials or plastic bags in a freezer. This procedure removes the inconvenience of having to thaw a large volume of frozen stock each time medium is prepared. Some have found that heating in a microwave oven is a satisfactory and quick method of thawing concentrated medium.

STERILIZATION OF MEDIA

Plant tissue culture media generally are sterilized by autoclaving at 121° C and 1.05 kg/cm^2 (15-20 psi). The time required for sterilization depends upon the volume of the medium in the vessel. The minimum times required for sterilization of different volumes of media are listed below. It is advisable to dispense media in small aliquots whenever possible because many media ingredients are broken down with prolonged exposure to heat and pressure. Media can be sterilized by heating in a microwave oven for 5 min or longer, depending on factors such as the microwave power, vessel type and volume of medium (Tisserat, et al., 1992; Youssef and Amin, 2001). Thermo-labile media ingredients should be filter-sterilized rather than autoclaved. Stock solutions of the heat-labile components are prepared, and then passed through a 0.45- or 0.22- µm filter into a sterile container. The filtered solution is then added with a sterile pipet to the culture medium, which has been autoclaved and allowed to cool to approximately 45°-50° C. The medium is then dispensed under sterile conditions.

POWDERED MEDIA

Only the finest available reagents are used in the preparation of powdered tissue culture media. All media are highly hygroscopic and must be stored in airtight containers. Once the bottle has been opened, the remaining contents should be protected from atmospheric moisture. Desiccation prolongs the storage period. Store at 2° to 6° C. Powdered media is available in 1-liter (1 L), 10 L, and 50 L units. Larger sizes are available for selected media and upon request for others.

Quality Control

All raw materials used in the production of powdered tissue culture media are held in quarantine until assayed according to appropriate standards. All raw materials must meet appropriate standards before they are accepted for use in the final product.

Random samples are withdrawn for quality control tests. Media are prepared to 1x concentration and are sterilized by autoclaving for a time appropriate to the volume. The following tests are preformed: growth promotion, solubility, appearance, and pH. Attempts to prepare concentrated media from powdered products are not recommended. Liquid media prepared in 5x to 10x concentrations from powdered products will often result in the insolubility of free-base amino acids or in the formation of salt complexes with resulting precipitation.

Custom Formulations

*Phyto*Technology Laboratories can provide custom formulations. Please allow 2 to 4 weeks for delivery after receipt of order and formula confirmation.

Prepared Powdered Media

Prepared media offers the following advantages over using media prepared from stock solutions.

1. Less weighing is required when making media from prepared powder media. This helps eliminate technician errors that can be made when preparing the final media.
2. Eliminates errors that occur in weighing or preparing concentrated stock solutions.
3. Homogenous powdered media ensures that all components of the media are included.
4. Lot-to-Lot consistency – *Phyto*Technology Laboratories manufactures all media following cGMP so that each batch of media is manufactured in the same manner every time.
5. Large Batch Availability – By reserving lots of media, you can use the same lot of media for an entire project or production cycle. This ensures uniformity in your production of plants.
6. Cost Savings – Most labs find that the use of powdered media actually results in cost savings in their media production operations, as less time is required to weigh out and prepare the media.

MEDIA PREPARATION

From Packaged Powder

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. Preparing the medium in a concentrated form is not recommended as some salts added to the medium may affect shelf life and storage conditions. The basic steps for preparing the culture medium are as follows:

1. Measure out approximately 90% of the final required 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 original container with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in Step 2.
4. Add desired heat stable supplements (e.g., sucrose, gelling agent, vitamins, auxins, cytokinins)
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH/ KOH or HCl.
7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi), 121° C, for the time period described under "Sterilization of Media".
10. Allow medium to cool prior to use.

*Heating may be required to bring powders into solution.

POWDERED MEDIA AND BASAL SALT MIXTURES ARE FOR LABORATORY USE ONLY. NOT FOR DRUG, HOUSEHOLD, OR OTHER USES.

MATERIALS NOT PROVIDED

Deionized tissue culture grade water
1 N Hydrochloric Acid (HCl) (Product No. H245)
1 N Sodium Hydroxide (NaOH) (Product No. S835)
1 N Potassium Hydroxide (KOH) (Product No. P682)
Auxins, cytokinins, carbohydrates, gelling agents, and other supplements listed in the Biochemicals Section.

STORAGE

Store dry medium at 2-6° C. Deterioration of powdered medium may be recognized by: 1) color variations; 2) granulation, clumping, or particulate matter throughout the powder; 3) insolubility; 4) pH change; or 5) inability to promote growth when properly used.

PRECIPITATION IN PLANT TISSUE CULTURE POWDERED MEDIA

Precipitates are known to occur, with time, in plant tissue culture media. These precipitates have been analyzed (Dalton, *et al.,* 1983). They are composed of small, pale yellow-white particles. Analysis of precipitates indicated a predominance of iron, phosphate, and zinc. The probable cause of the precipitates is the inevitable oxidation of ferrous ions. There are no reports of detrimental effects on growth and development in plant tissue culture due to the precipitates.

From Basal Salt Solutions

Liquid 10x solutions are offered for your convenience. To avoid precipitation over long-term storage, *Phyto*Technology Laboratories has formulated two solutions which, when mixed at the proper dilution, makes a solution with the appropriate salt concentration. The basic steps for preparing 1 liter of culture medium are listed below.

CAUTION:
Basal salt solutions are supplied in non-autoclavable bottles.
If autoclaved, the bottles will melt.

1. Measure out approximately 700 mL of tissue culture grade water.
2. While stirring the water, add 100 mL of Macronutrient Solution (Product No. M654)
3. Continue stirring the mixture while adding 100 mL of Micronutrient Solution (Product No. M529)
4. Add desired heat stable supplements (e.g., sucrose, gelling agent, vitamins, auxins, cytokinins)
5. Add additional tissue culture grade water to bring the medium to the final volume of 1 liter.
6. While stirring, adjust medium to desired pH using NaOH, HCl or KOH.
7. If gelling agent is used, heat while stirring until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat-labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi), 121° C, for the time period described under Sterilization of Media.
10. Allow medium to cool prior to use.

BASAL SALT SOLUTIONS ARE FOR LABORATORY USE ONLY. NOT FOR DRUG, HOUSEHOLD, OR OTHER USES.

MATERIALS NOT PROVIDED

Deionized tissue culture grade water
1 N Hydrochloric Acid (HCI) (Product No. H245)
1 N Sodium Hydroxide (NaOH) (Product No. S835)
1 N Potassium Hydroxide (KOH) (Product No. P682)
Medium additives as required

STORAGE

Store basal salts at 2-6° C. Deterioration of basal salt solutions may be recognized by: 1) color change; 2) pH change; 3) precipitation of components; or 4) inability to promote growth when properly used.

VITAMIN PREPARATION AND USE

Vitamin Mixtures

Powdered vitamin mixtures are hygroscopic and must be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. The basic steps for preparing 1000x concentrated solutions with vitamin mixtures are listed below:

1. Measure out 70% of the final required volume of deionized/ distilled water (e.g., 70 mL for a final volume of 100 mL).
2. While stirring the water add the powdered vitamin mixture. Stir until completely dissolved. Increasing the pH and/or warming the solution (e.g., 35-37° C) may be required.
3. Rinse the original container with a small volume of water to remove traces of the powder. Add to the solution in Step 2.
4. Add additional water to bring the medium to the final volume.
5. The resulting 1000x concentrated solution should be used at a concentration of 1 mL/L of medium.
6. Follow the same steps to prepare a 100x concentrated solution and use at 10 mL/L of medium.

Vitamin Solutions

1. The vitamin solutions are sterile filtered through a double 0.2 µm filtration unit and are ready for use.
2. Vitamin solutions (1000x) should be added at a concentration of 1 mL/L of medium and vitamin solutions (100X) should be added at a concentration of 10 mL/L of medium to prepare the final recommended concentration of vitamins in the medium.

MATERIALS NOT PROVIDED

Basal media and additives as required
Deionized tissue culture grade water
1 N Hydrochloric Acid (HCl) (Product No. H245)
1 N Sodium Hydroxide (NaOH) (Product No. S835)
1 N Potassium Hydroxide (KOH) (Product No. P682)

VITAMIN MIXTURES AND SOLUTIONS ARE FOR LABORATORY USE ONLY.
NOT FOR DRUG, HOUSEHOLD, OR OTHER USES.

STORAGE

Store vitamin mixtures and solutions in a refrigerator at 2-6° C. Vitamin solutions may develop precipitates during storage; the most common occurrence of this due to the high concentration of *myo*-inositol in many of the 1000x solutions. This can be redissolved without any detrimental effects to the vitamin's quality by warming the solution in a water bath (35-37° C) for a short period of time.

PREPARATION OF PHYTOPATHOLOGY MEDIA

Bacteria Screening Medium 523 (Product No. B129)

This medium is a slight modification of Viss et al. (1991) containing 8 g/L agar rather than the 10 g/L in the original published formulation. This is a rich medium containing sucrose as the energy source, vitamins from yeast extract, and amino acids from hydrolyzed casein. Bacteria Screening Medium 523 was used in a screening protocol published by Thomas (2004).

1. Suspend 32.15 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil while stirring. Avoid overheating this product as it might result in a softer agar gel and increased darkening of the medium.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving) or as indicated in an appropriate protocol.

Corn Meal Agar (Product No. C442)

Corn Meal Agar has been used for many years as a general purpose medium for culturing various fungi. It is a simple medium consisting of corn meal and agar; 1% glucose may be added as an energy source to enhance fungal growth. The basic steps for preparing this product is listed below.

1. Suspend 22.0 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil while stirring; the corn meal will not dissolve. Avoid overheating this product as it might result in a softer agar gel and increased darkening of the medium.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Czapek–Dox Broth (Product No. C443)
Czapek–Dox Agar (Product No. C506)

Czapek–Dox Broth and Agar are used for the cultivation of fungi and soil-borne bacteria. Sucrose is the sole energy source; nitrate is the nitrogen source.

1. Suspend the appropriate amount of powder in 1 liter of distilled/ deionized water. Dissolve the agar medium by heating to a boil while stirring. Avoid overheating this product as it might result in a softer agar gel and increased darkening of the medium.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Leifert & Waites Sterility Test Medium (Product No. L476)

This medium was developed by Leifert and Waites (1992) to determine and identify potential pathogens in tissue culture explants. Explants are placed in the medium for 1-2 weeks (or longer depending on the indexing protocol being followed). The medium was developed to contain enough nutrients to support the maintenance of the explant but also contains nutrients required to support the growth of any microorganisms in the explant.

1. Suspend the 45.22 g of powder in 1 liter of distilled/ deionized water. Dissolve the medium by stirring. If agar is desired add it and adjust the pH as desired. Dissolve the agar by heating to a boil while stirring. Avoid overheating this product as it might result in a softer agar gel and increased darkening of the medium.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving) or as directed in an appropriate indexing procedure, e.g., Procedure 44.1, Indexing Stage 1 Shoot Cultures (Kane, 2000).

Malt Extract Broth (Product No. M484)
Malt Extract Agar (Product No. M498)

Malt Extract Broth and Agar are used for the isolation and detection of yeasts and molds. The carbohydrates present in these media are well studied to support the growth of many fungi. The basic steps for preparing these products are listed below.

1. Suspend the appropriate amount of powder in 1 liter of distilled/ deionized water. Dissolve the agar medium by heating to a boil while stirring. Avoid overheating this product as it might result in a softer agar gel and increased darkening of the medium.

2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Nutrient Broth (Product No. N611)
Nutrient Agar (Product No. N601)

*Phyto*Technology Laboratories Nutrient Broth and Agar are general purpose microbiological media for less fastidious organisms. The basic steps for preparing these products are listed below:
1. Suspend the appropriate amount of powder in 1 liter of distilled/ deionized water. Dissolve the agar medium by heating to a boil.
2. Sterilize the medium in a validated autoclave at 1 kg/ cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Oatmeal Agar (Product No. O606)

Oatmeal agar is typically used to culture fungi, particularly for macrospore formation. The oatmeal supplies the nitrogen, carbon, protein, and nutrients.
1. Suspend 10.5 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil while stirring; the oatmeal will not dissolve.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Potato Dextrose Broth (Product No. P762)
Potato Dextrose Agar (Product No. P772)

Potato Dextrose Broth and Agar are standard media used for culturing bacteria, fungi, and yeast. The basic steps for preparing this product are listed below:
1. Suspend the appropriate amount of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Wilkins─Chalgren Agar (Product No. W887)

Wilkins─Chalgren Agar is typically used for the cultivation of anaerobes. Wilkins and Chalgren (1976) developed this medium to determine the minimum inhibitory concentration (MIC) of antibiotics on anaerobic bacteria using the agar dilution method.
1. Suspend 43.0 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

YEP Medium (Product No. Y889)

YEP Medium has been used to culture *Agrobacterium* and yeasts.
1. Suspend 40.0 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil.
2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

YMB Medium (Product No. Y893)

YMB Medium has been used to culture *Rhizobium* and various fungi.
1. Suspend 26.2 g of powder in 1 liter of distilled/ deionized water. Dissolve the agar by heating to a boil.

2. Sterilize the medium in a validated autoclave at 1 kg/cm^2 (15 psi) 121° C. The medium should attain a temperature of 121° C for at least 15 minutes. Refer to the section on the Sterilization of Media.
3. Dispense the medium as desired (before or after autoclaving).

Storage of Phytopathology Media

All medium preparations should be stored at 2-6° C. Store dry medium at room temperature (below 30° C) or refrigerated (2-6° C) as indicated on the product label. The dehydrated powder should be free flowing. Deterioration of the powdered medium may be recognized by: 1) granulation, clumping, or particulate matter throughout the powder; 2) pH change; or 3) inability to promote microbial growth when properly used, i.e., with control cultures in an validated protocol. Consult the certificate of analysis (available on the *Phyto*Technology Labs' web site) for details concerning the appearance of the powder, pH, and solubility.

ANTIBIOTIC SELECTION, PREPARATION, AND STORAGE

In general, antibiotics require storage in a refrigerator or freezer. Aminoglycosides (e.g., Kanamycin) are hygroscopic and should be stored in a desiccator. Storage of many powdered antibiotics at -20° C is not recommended and increases the risk of water condensation. All antibiotics should be protected from direct sunlight. Rifampicin and Amphotericin B are very sensitive to light and should be stored in the dark.

The relationship between the weight (mg) of antibiotic, the activity of the powder (µg/mg or units per mg), the volume of solution to prepare (mL), and the concentration (µg/mL) of antibiotic desired in the solution is:

$$\text{Weight} = \frac{\text{Volume X Concentration}}{\text{Activity}}$$

Most antibiotic solutions will remain stable stored at -0° C for up to 3 months or longer (unless otherwise noted on the following table). However, Rifampicin and Tetracyline should be freshly prepared for each use. Most antibiotics are heat liable and should be filter sterilized using membranes that minimize absorption (e.g., cellulose acetate).

ANTIBIOTIC/ ANTIMYCOTIC/ SELECTION AGENT GUIDE

Product	Prod. No.	Mol. Wt.	Gram (+) Bacteria	Gram (-) Bacteria	Mycobacteria	Fungi	Yeast	Mycoplasma	Selection Agent	Microbe Toxicity (µg/mL)	Toxicity to Plant Tissues[1] (ug/mL)	Solubility	Store Solution[2]
Amoxicillin	A122	419.5	+	++						Varies	-	Water	N/A
Amphotericin B	A119	924.1				++	++			2.5	>5	DMSO	R
Ampicillin	A116	371.4	++	++					++	50	100	Water	F
Bacitracin	B132	1421.6	++							50	150	Water	F
Butirosin Disulfate	B145	751.7	++	++					++	25-100	-	Water	N/A
Carbenicillin	C346, C540	422.4	+	++						500	>1000	Water	F
Cefotaxime	C380, C537	477.4	+	++						90	>100	Water	F
Chloramphenicol	C252	323.1	++	++	+			+	++	128	1-64	EtOH	R
Erythromycin	E344	733.9	++	++						0.5-30	150	Water	R
G418	G810	692.7							++	NA	50	Water	R
Gentamicin	G570	575.7	+	++				++		50	80	Water	R
Hygromycin B	H370, H385, H397	527.5							++	NA	20-400	Water	R
Kanamycin	K378, K586	582.6	++	++				++	++	100	2	Water	R
Kasugamycin	K559	433.8	+	+		++				75	-	Water	N/A
Neomycin	N584	908.9	++	++					++	50	900	Water	R
Nystatin	N581	926.1				++	++			50	40	Not soluble	F
Oxacillin	O667	441.4								25	-	Water	F
Paromomycin	P710	713.7	++						++	30	50	Water	R
Penicillin G	P777	356.4	++	+						Varies	100	Water	F

Product	Prod. No.	Mol. Wt.	Gram (+) Bacteria	Gram (-) Bacteria	Mycobacteria	Fungi	Yeast	Mycoplasma	Selection Agent	Microbe Toxicity (µg/mL)	Toxicity to Plant Tissues[1] (ug/mL)	Solubility	Store Solution[2]
Rifampicin	R501	822.9	++	++	++					15	100	Water (Slight)	F
Spectinomycin	S742	405.3	+	++					++	20	500	Water	F
Streptomycin	S739	1457	++	++						100	16	Water	F
Tetracycline	T859	480.9	++	++						10	50	Water	F[3]
Timentin	T869, T767	NA	++	++					++	64+	200	Water	F
Tobramycin	T834	1425.5	+	++						8	-	Water	R
Vancomycin	V870	1485	++							5	80	Water	R

- = Unknown or highly variable
++ = Effective against most microorganisms
+ = Effective against certain microorganisms

[1]Plants exhibit great differences between species in toxic sensitivity to antibiotics. A concentration showing no toxicity to one plant species may exceed the toxic concentration in a different species. For this reason, the concentrations for plant toxicity noted in the table may be higher or lower for different plant species.

[2]Solution storage: F = Freezer; R = Refrigerator; RT = Room Temperature; N/A = Stability data not available.

[3]Aqueous solutions of Tetracycline hydrolyze (even if frozen) yielding a hazy appearance. Solutions should only be stored (frozen) for short periods, e.g., one week. Preparation of fresh solutions is recommended.

Carbenicillin (Product Nos. C346 & C540)

Carbenicillin is a white to off-white, hygroscopic powder that is soluble in either water or alcohol. Carbenicillin is most effective against gram-negative bacteria but may also have some effect against gram-positive bacteria. Aqueous solutions of Carbenicillin are reported to be stable for up to 24 hours at room temperature and for up to 72 hours when stored at 2-6° C.

Cefotaxime (Product Nos. C380 & C537)

Cefotaxime is a white to off-white powder, which is freely soluble in water. Variations in color of the freshly prepared solutions do not necessarily indicate changes in potency. Store this product in an airtight container protected from light. Aqueous solutions of Cefotaxime at a pH of 4.5-6.2 are stable for 14-21 days when stored at 2-6° C. Cefotaxime is most effective against gram-negative bacteria.

Geneticin® [Antibiotic G418] (Product No. G810)

Although it is related to Gentamicin, Geneticin is not normally used as a standard antibiotic. Its most common application is in molecular biology as a selection agent. Geneticin, also known as antibiotic G418 sulfate, is toxic to bacteria, yeast, protozoa, helminthes, and mammalian cells. Resistance is conferred by one of two dominant genes of bacterial origin, which can also be expressed in eukaryotic cells.
Geneticin is water-soluble and can be stored at room temperature for as long as 1 year. Aqueous solutions should be stored frozen. The amount of Geneticin required for selection will vary with each cell type and growth cycle. Although cells that are multiplying will be affected sooner than those that are not, cells that are in log phase will still require 3 to 7 days for selection.
Geneticin® is a registered trademark of Invitrogen, Inc.

Hygromycin B (Product Nos. H370, H385, & H397)

Hygromycin B is an aminoglycoside antibiotic, which is effective against prokaryotic and eukaryotic microorganisms and cells. Similar to Geneticin®, its most common application is in molecular biology as a selection agent. Cells transformed with the hph gene are resistant to Hygromycin B.

Hygromycin B is provided as a 100 mg/mL aqueous solution with an average potency of 1,000 units/mg. The recommended concentration range for use as a selection agent is 10 - 400 µg/mL. Stock solutions can be stored for 1 year at 2-6° C; solutions should NOT be frozen as this can reduce their potency.
Typical selection concentrations:

Prokaryotes – 100 µg/mL
Lower eukaryotes – 200 µg/mL
Higher eukaryotes – 150-400 µg/mL

ANTIOXIDANT MIXTURE
(Product No. A126)

The antioxidant mixture is intended to reduce the browning of plant tissue prior to or during culture. The product should be prepared immediately before use. Short-term storage at -20° is acceptable. The product is supplied as a powder in packages that prepare 1 or 10 liters of antioxidant solution. If other volumes are desired the product can be prepared at 250 mg/L. When prepared at this concentration no further dilution is typically necessary. The solution can be sterilized by filtration through a 0.22 µm sterile membrane; the solution should not be autoclaved. Tissue can be placed in the solution for 5 - 30 minutes prior to placement on fresh medium.
The antioxidant mixture contains:
100 mg/L L-Ascorbic Acid, Free Acid
150 mg/L Citric Acid, Free Acid, Anhydrous

USE AND PREPARATION OF BANANA POWDER
(Product No. B852)

*Phyto*Technology Laboratories offers banana powder for use in orchid and other plant cell cultures. Product number B852 is a powder from a spray-dried mixture of banana and maltodextrin. This product is typically used between 30 – 50 g/L. To reduce clumping, add powder slowly to the culture medium with constant stirring. The presence of banana solids is common in medium containing this product.

USE AND STORAGE OF COCONUT WATER
(Product No. C195)

Coconut water has been shown to stimulate shoot proliferation in many species of plants. Coconut water is prepared from selected coconuts and processed to remove most of the protein. Remaining protein levels in the water may vary from one lot to the next and may result in the formation of precipitates when the product is frozen. This precipitation should not affect the growth of the plant tissue. The precipitate can be removed by filtering or by allowing it to settle to the bottom of the bottle and then decanting. Coconut water can be divided into smaller aliquots, corresponding to your standard medium batch size, and refrozen until needed. Coconut water should be used at a concentration of 5-20% (v/v).

GELLING AGENTS

Agar has long been used to solidify media for plant tissue culture. The type of agar or gelling agent used can influence the growth of the tissue in culture. Both purity and cost of the gelling agent are important factors in any research or production operation. *Phyto*Technology has expanded its line of gelling agents to allow greater selection in choosing the plant cell culture tested gelling agent for your particular requirement. Listed in the following table are some criteria, which may also help you select the appropriate product for your application.

GELLING AGENTS CHART

Prod. No.	Prod. Name	Appearance of Powder/ Gel	Gel Strength (g/cm^2)	Typical Use (g/L)
A111	Agar, Microprop. Grade from *Gracilaria*	White-Off White/Opaque	≥900	5.0 to 8.0
A296	Agar, Microprop. Grade from *Gelidium*	Tan/Tan	700-950	8.0 to 10.0

GELLING AGENTS CHART (cont.)				
Prod. No.	**Prod. Name**	**Appearance of Powder/ Gel**	**Gel Strength (g/cm^2)**	**Typical Use (g/L)**
A181	Agar, Research Grade from *Gelidium*	Tan/Tan-Straw Colored	700-1050	8.0 to 10.0
A175	Agar, Purified Grade from *Gelidium*	White/Clear- Opaque	700-1200	8.0 to 10.0
A133	Agargellan, Blend of Agar & Gellan Gum	Off White-Tan/ Clear- Opaque	NSE*	3.5 to 5.0
A105	Agarose, Low Gelling Temp.	White-Off White/ Clear-Slightly Hazy	≥ 500	5.0 to 10.0
A110	Agarose, High Gelling Temp.	White-Off White/ Clear-Slightly Hazy	NSE*	5.0 to 10.0
A108	Alginic Acid, Sodium Salt (Sodium Alginate)	Yellow-Beige/ Clear- Opaque	NSE*	See Protocol Below
C257	Carrageenan	Off White-Tan/ Tan-Straw Opaque	NSE*	7.0 to 10.0
G434	Gellan Gum, Biotech Grade	White-Off White/ Clear- Slightly Hazy	≥ 800	1.5 to 2.5

*NSE = No Specification Established

Preparation of Agargellan™ (Product No. A133)

Agargellan is a proprietary blend of micropropagation grade agar and Biotech grade CultureGel (Gellan Gum) that was developed to help control hyperhydricity in plant tissue cultures. Agargellan provides the positive attributes of both agar and Gellan Gum and is superior to Gellan Gum alone in applications where hyperhydricity is a problem. It also serves as an economical alternative to agar for many species. Agargellan produces a semi-clear gel which allows for improved detection of contamination, relative to agar gels. Agargellan should be used at a concentration of 3.5 - 5.0 g/L. As with agar and Gellan Gum, is should be added to stirring medium that is room temperature.

Preparation of Alginate Gel/Beads (Product No. A108)

Alginic Acid has been used for a number of cell and tissue culture applications including use as a physical support similar to agarose and for the preparation of gelled beads. Both of these applications have been used to immobilize and embed suspension cells and protoplasts (Adaoha Mbanaso and Roscoe, 1982; Chee and Cantliffe, 1989; Draget *et al.*, 1988; Larkin *et al.*, 1988). Alginate solutions form a reversible gel at room temperature in the presence of calcium ions. The gel can be re-liquified with a chelating agent, such as citrate. Cells imbedded in gel matrices can be manipulated with significantly less physical damage during handling than cells in liquid medium.

Alginate should be dissolved in a low calcium (e.g., 2 mM) buffered medium at 1.75 - 4.0% (w/v). If protoplasts are to be embedded in the gel then the medium should contain an appropriate osmoticum. Alginic acid will require several hours to dissolve. As it dissolves, the solution will increase in viscosity. This viscosity will negate filter sterilization through a 0.2 µm membrane; a 0.45 µm membrane can be used. While some researches have indicated that alginate solutions can be autoclaved, Larkin et al. (1988) noted a reduction in bead-making capacity with increased autoclave time.

If protoplasts are to be embedded, they should first be concentrated by centrifugation in a low calcium medium and added to alginate at an appropriate density (e.g., 1 x 10^5 cells/ml). The protoplast-alginate solution is added drop-wise to a solution containing 50 mM $CaCl_2$ and an appropriate osmoticum. Each droplet will for a bead. The beads should remain in the $CaCl_2$ for up to 45 minutes to ensure optimum gel matrix formation.

Preparation of CultureGel™ Gellan Gums (Product No. G434)

CultureGel Gellan Gum is an alternative gelling agent to agars. Gellan Gum is produced from a bacterial substrate composed of glucuronic acid, rhamnose, and glucose. G434 is a biotech grade that is very clear that produces high strength gel that is significantly clearer than agar gels. This aids in the detection of microbial contamination. Gellan gum offers an economical alternative to agar in many *in vitro* applications. Gellan gum is typically used at a concentration from 1.5 - 2.5 g/L.

PLANT GROWTH REGULATORS

The importance of plant growth regulators in plant tissue culture is well documented. *Phyto*Technology offers a broad range of plant growth regulators specifically tested for plant cell culture. Each product is assayed for physical and chemical characteristics then is biologically tested following the criteria established for powdered media. Each auxin is tested for enhancement of callus growth and/or root initiation *in vitro*. Each cytokinin is tested for stimulation of shoot production.

PLANT GROWTH REGULATORS ARE FOR LABORATORY USE, PLANT TISSUE CULTURE MEDIA PREPARATION, AND PLANT RESEARCH PURPOSES ONLY.
THEY ARE NOT FOR USE AS PLANT GROWTH REGULATORS ON DEVELOPED PLANTS.
THEY ARE NOT FOR DRUG OR HOUSEHOLD USE.

Product Use

Auxins: Auxins are generally used in plant cell culture at a concentration range of 0.01-10.0 mg/L. When added in appropriate concentrations they may regulate cell elongation, tissue swelling, cell division, formation of adventitious roots, inhibition of adventitious and axillary shoot formation, callus initiation and growth, and induction of embryogenesis.

Cytokinins: Cytokinins are generally used in plant cell culture at a concentration range of 0.1-10.0 mg/L. When added in appropriate concentrations they may regulate cell division, stimulate auxiliary and adventitious shoot proliferation, regulate differentiation, inhibit root formation, activate RNA synthesis, and stimulate protein and enzyme activity.

Gibberellins: Gibberellins are generally used to promote stem elongation, flowering, and breaking dormancy of seeds, buds, corms, and bulbs. There are over 90 forms of gibberellins, but GA_3 is the most commonly used form. Compounds like paclobutrazol and ancymidol inhibit the synthesis of gibberellins.

Abscisic Acid: Abscisic Acid (ABA) plays a role in dormancy development in embryos, buds and bulbs, and in leaf abscission. When used in tissue culture, ABA inhibits the growth of shoots and the germination of embryos. Fluridone may inhibit ABA synthesis.

Polyamines: Polyamines are compounds that occur in high levels within plants and are used in tissue culture media at concentrations of 10-1000 mM. Polyamines may enhance regeneration of roots, shoots and embryos, delay or prevent senescence, and regulate flowering.

Methods Of Preparation

To prepare a 1 mg/mL stock solution: Add 100 mg of the plant growth regulator to a 100 mL volumetric flask or other glass container. Add 3-5 mL of solvent to dissolve the powder. Once completely dissolved, bring to volume with distilled/ deionized water. Stirring the solution while adding water is recommended to keep the material in solution. Store the stock solution as recommended in the tables. One mL of the stock solution in 1 liter of medium will yield a final concentration of 1.0 mg/L of the plant growth. (See conversion tables).

$$\frac{\text{Desired Hormone Concentration} \times \text{Medium Volume}}{\text{Stock Solution Concentration}} = \text{Volume of Stock Solution Required}$$

Stock Solution Dilution Chart

To use this chart:

1. Determine the final concentration of the hormone/ vitamin etc. desired in the culture medium. In column A, locate the final concentration desired under the heading corresponding to the quantity of medium you will prepare.

2. Once you have located the desired final concentration then go across the chart to column B to determine the concentration of stock solution to prepare.

3. Find the volume of stock solution to use to achieve the final desired concentration in the medium in column C.

B Concentration of Stock Solution	C Amount to use (mL)	A Concentration of Final Solution (mg/L)				
		250 mL	500 mL	1 L	2 L	10 L
0.01 mg/mL	0.1	0.004	0.002	0.001	0.0005	0.0001
	0.5	0.02	0.01	0.005	0.025	0.005
	1.0	0.04	0.02	0.01	0.005	0.001
	10.0	0.4	0.2	0.1	0.05	0.01
0.1 mg/mL	0.1	0.04	0.02	0.01	0.005	0.001
	0.5	0.2	0.1	0.05	0.25	0.05
	1.0	0.4	0.2	0.1	0.05	0.01
	10.0	4.0	2.0	1.0	0.5	0.1
1.0 mg/mL	0.1	0.4	0.2	0.1	0.05	0.01
	0.5	2.0	1.0	0.5	0.25	0.5
	1.0	4.0	2.0	1.0	0.5	0.1
	10.0	40.0	20.0	10.0	5.0	1.0
10.0 mg/mL	0.1	4.0	2.0	1.0	0.5	0.1
	0.5	20.0	10.0	5.0	2.5	0.5
	1.0	40.0	20.0	10.0	5.0	1.0
	10.0	400.0	200.0	100.0	50.0	10.0

PGR Class	Product Name	Product Number	Function in Plant Tissue Culture
Auxins	Indole-3-Acetic Acid Indole-3-Butyric Acid Indole-3-Butyric Acid, K-Salt α-Naphthaleneacetic Acid α-Naphthaleneacetic Acid, K-Salt 2,4-D (Solutions) ρ-Chlorophenoxyacetic acid Picloram Dicamba	I885/I364 I538/I460 I530 N600/N605 N610 D295/D301 C213 P717 D159/D165	Adventitious root formation (high concentration) Adventitious shoot formation (low concentration) Induction of somatic embryos Cell Division Callus formation and growth Inhibition of axillary buds Inhibition of root elongation
Cytokinins	6-Benzylaminopurine 6-(γ,γ-Dimethylallylamino)purine (2iP) 2iP-2HCl Kinetin Thidiazuron (TDZ) N-(2-Chloro-4-pyridyl)-N-phenylurea Zeatin Zeatin Riboside	B800/B130 D525/D217 D341 K750/K483 T888 C279 Z125/Z860 Z899/Z875	Adventitious shoot formation Inhibition of root formation Promotes cell division Modulates callus initiation and growth Stimulation of axillary bud breaking and growth Inhibition of shoot elongation Inhibition of leaf senescence
Gibberellins	Gibberellic Acid (GA_3) $GA_{4/7}$	G500/G198 G358	Stimulates shoot elongation Release seeds, embryos, and apical buds from dormancy, Inhibits adventitious root formation
Abscisic Acid	Abscisic Acid	A102	Stimulates bulb and tuber formation Stimulates the maturation of embryos Promotes the start of dormancy, leaf abscision
Polyamines	Putrescine Spermidine	P733 S837	Promotes adventitious root formation Promotes somatic embryogenesis Promotes shoot formation
Antimitotics	Colchicine Oryzalin Trifluralin	C226 O630 T828	Binds to the tubulin dimers during cell division thus preventing the formation of spindle fibers; this results in doubled chromosomes

Continued next page

PGR Class	Product Name	Product Number	Function in Plant Tissue Culture
Dwarfing Agents/ "Anti-GA's"	Ancymidol CCC Paclobutrazol Trinexapac-Ethyl	A123 C207 P687 T761	Interferes with gibberellin synthesis or activity Reduces internodal elongation Promotes tuber, corm, and bulb formation

PLANT GROWTH REGULATORS
CONCENTRATION/ MOLARITY (µM) CONVERSIONS AND HANDLING RECOMMENDATIONS

Prod. No	Growth Regulator	Mol. Wt.	CAS No.	mg/L to PGR µM Conversion[1] (See explanation below table)						Solvent[2]	Dilute In	Store Prod.	Store Stock Soln.	Sterilize By	Typical Working Conc. (mg/L)
				0.1	0.3	0.5	0.7	0.9	1.0						
A102	ABA	264.3	1437545-2	0.38	1.1	1.9	2.65	3.4	3.8	KOH	Water	F	F	CA/F	0.1-10.0
A120	Adenine	135.1	73-24-5	0.74	2.2	3.7	5.18	6.7	7.4	1 N HCl	Water	RT	C	CA	50-250
A545	Adenine Hemisulfate	404.4	321-30-2	0.25	0.7	1.2	1.73	2.2	2.5	Water	Water	RT	C	CA	50-250
A123	Ancymidol	256.3	12771-68-5	0.39	1.2	2	2.73	3.5	3.9	DMSO	Water	C	F	CA/F	1.0-10.0
A147	Auxindole™	269.9	N/A	0.37	1.1	1.8	2.59	3.3	3.7	EtOH	Water	F	F	F	0.003-3.0
B148	4-Bromophenoxy-acetic Acid	231.1	1878-91-7	0.43	1.3	2.2	3.02	3.9	4.3	KOH	Water	RT	C	F	0.01-5.0
B155	Bromoxynil	276.9	1689-84-5	0.36	1.1	1.8	0.25	3.3	3.6	DMSO	Water	RT	F	F	—
B151	N6-Benzoyladenine	239.2	4005-49-6	0.42	1.3	2.1	2.94	3.8	4.2	DMSO	Water	C	F	F	0.1-10.0
B202	BPA	309.4	2312-73-4	0.32	1	1.6	2.26	2.9	3.2	EtOH	Water	F	F	CA/F	0.1-5.0
B800	BA	225.3	1214-39-7	0.44	1.3	2.2	3.11	4	4.4	KOH	Water	RT	C	CA	0.1-5.0
B130	BA Solution, 1 mg/mL	225.3	1214-39-7	0.44	1.3	2.2	3.11	4	4.4	N/A	Water	C	C	CA	0.1-5.0
C207	CCC	158.1	999-81-5	0.63	1.9	3.2	4.43	5.7	6.3	Water	Water	RT	C	CA	Up to 500
C213	4-CPA	186.6	122-88-3	0.54	1.6	2.7	3.75	4.8	5.4	EtOH	Water	RT	C	CA	0.1-10.0
C279	4-CPPU	247.7	68157-60-8	0.4	1.2	2	2.82	3.6	4	DMSO	Water	C	C	F	0.001-1.0
C283	t-Cinnamic Acid	148.2	140-10-3	0.68	2	3.4	4.72	6.1	6.7	KOH	Water	RT	C	CA	0.1-10.0
C226	Colchicine	399.4	64-86-8	0.25	0.8	1.3	1.75	2.3	2.5	Water	Water	RT	C	F	—
D159	Dicamba	221	1918-00-9	0.45	1.4	2.3	3.17	4.1	4.5	EtOH	Water	C	C	CA	0.01 to 10.0
D165	Dicamba Solution, 1 mg/mL	221	1918-00-9	0.45	1.4	2.3	3.17	4.1	4.5	N/A	Water	C	C	CA	0.01 to 10.0
D297	Dikegulac	274.3	18467-77-1	0.36	1.1	1.8	2.52	3.2	3.6	EtOH	Water	C	C	F	0.05-10.0
D295	2,4-D Solution, 1 mg/mL	221	94-75-7	0.45	1.4	2.3	3.17	4.1	4.5	N/A	Water	C	C	CA	0.01-5.0
D309	2,4-D Solution, 10 mg/mL	221	94-75-7	0.45	1.4	2.3	3.17	4.1	4.5	N/A	Water	C	C	CA	0.01-5.0
D525	2iP	203.2	2365-40-4	0.49	1.5	2.5	3.44	4.4	4.9	KOH	Water	F	F	CA	1.0-30.0
D217	2iP Solution, 1 mg/mL	203.2	2365-40-4	0.49	1.5	2.5	3.44	4.4	4.9	N/A	Water	F	F	CA	1.0-30.0
D341	2iP•2HCl	276.2	N/A	0.36	1.1	1.8	2.5	3.3	3.6	Water	Water	F	F	CA	1.0-30.0
E348	Ethephon	144.5	16672-87-0	0.69	2.1	3.5	4.84	6.2	6.9	Water	Water	RT	C	F	0.01-10.0
F357	Fluridone	329.3	59756-60-4	0.30	0.9	1.5	2.13	2.7	3.0	DMSO	Water	C	C	F	—
F376	Flurprimidol	312.3	56425-91-3	0.32	1	1.6	2.24	2.9	3.2	EtOH/ DMSO	Water	C	C	F	0.01-5.0
G345	Glyphosate	169.1	1071-83-6	0.59	1.8	3	4.13	5.3	5.9	KOH	Water	RT	C	F	0.01-5.0
G500	GA_3	346.4	77-06-5	0.29	0.9	1.4	2.02	2.6	2.9	EtOH	Water	RT	C	CA/F	0.01-5.0
G198	GA_3 Solution, 1 mg/mL	346.4	77-06-5	0.29	0.9	1.4	2.02	2.6	2.9	N/A	Water	C	C	CA/F	0.01-5.0

G358	Gibberellins A_4+A_7	N/A	N/A	N/A						EtOH	Water	C	F	CA/F	0.01-5.0

Continued next page

Prod. No	Growth Regulator	Mol. Wt.	CAS No.	mg/L to PGR µM Conversion[1] (See explanation below table)						Solvent[2]	Dilute In	Store Prod.	Store Stock Soln.	Sterilize By	Typical Working Conc. (mg/L)
				0.1	0.3	0.5	0.7	0.9	1.0						
I885	IAA	175.2	87-51-4	0.57	1.7	2.9	3.99	5.1	5.7	KOH	Water	F	F	CA/F	0.01-3.0
I364	IAA Solution, 1 mg/mL	175.2	87-51-4	0.57	1.7	2.9	3.99	5.1	5.7	N/A	Water	F	F	CA/F	0.01-3.0
I538	IBA	203.2	133-32-4	0.49	1.5	2.5	3.44	4.4	4.9	KOH	Water	C	F	CA/F	0.1-10.0
I460	IBA Solution, 1 mg/mL	203.2	133-32-4	0.49	1.5	2.5	3.44	4.4	4.9	N/A	Water	F	F	CA/F	0.1-10.0
I560	IBA K-Salt	241.3	60096-23-3	0.41	1.2	2.1	2.9	3.7	4.1	Water	Water	C	F	CA/F	0.1-10.0
I409	IPA	189.2	830-96-6	0.52	1.6	2.6	3.69	4.8	5.3	KOH	Water	F	F	CA/F	0.1-10.0
P717	Picloram	241.5	2/1/1918	0.41	1.4	2.1	2.9	3.7	4.1	DMSO	Water	RT	C	CA	0.01-10.0
J379	Jasmonic Acid	210.3	3572-66-5	0.47	1.4	2.4	3.32	4.3	4.8	EtOH	NA	C	F	F	0.01-100.0
J389	Methyl Jasmonate	224.3	39924-52-2	.0.44	1.3	2.2	224	4	4.5	EtOH	NA	RT	F	F	0.01-100.0
K750	Kinetin	215.2	525-79-1	0.46	1.4	2.3	3.25	4.2	4.7	KOH	Water	F	F	CA/F	0.1-5.0
K438	Kinetin Solution, 1 mg/mL	215.2	525-79-1	0.46	1.4	2.3	3.25	4.2	4.7	N/A	Water	F	F	CA/F	0.1-5.0
M494	Maleic Acid Hydrazide	112.1	123-33-1	0.89	2.7	4.5	6.24	8	8.9	KOH	Water	RT	C	F	0.01-10.0
N600	NAA	186.2	86-87-3	0.54	1.6	2.7	3.76	4.8	5.4	KOH	Water	RT	C	CA	0.1-10.0
N605	NAA Solution, 1 mg/mL	186.2	86-87-3	0.54	1.6	2.7	3.76	4.8	5.4	N/A	Water	C	C	CA	0.1-10.0
N610	K-NAA	224.3	15165-79-4	0.44	1.3	2.2	3.12	4	4.5	Water	Water	RT	C	CA	0.1-10.0
N564	β-Naphthoxy-acetic Acid	202.2	120-23-0	0.49	1.5	2.5	3.48	4.5	5.0	KOH	Water	RT	C	CA	0.1-10.0
O630	Oryzalin	346.4	19044-88-3	0.29	0.9	1.4	2.02	2.6	2.9	DMSO	Water	RT	C	CA	—
P687	Paclobutrazol	293.8	76738-62-0	0.34	1.0	1.7	2.4	3.1	3.4	DMSO	Water	RT	C	CA/F	—
P694	Phloroglucinol	126.1	6009-90-7	0.79	2.4	4	5.55	7.1	7.9	Water	Water	RT	C	CA/F	up to 162
P717	Picloram	241.5	1918-02-1	0.41	1.2	2.1	2.90	3.7	4.1	DMSO	Water	RT	C	CA/F	0.01-10.0
P733	Putrescine	161.1	333-93-7	0.62	1.9	3.1	4.35	5.6	6.2	Water	Water	RT	C	F	—
S837	Spermidine	145.3	124-20-9	0.69	2.1	3.4	4.82	6.2	6.9	Water	Water	C	C	F	—
S746	SADH	160.2	1596-84-5	0.62	1.9	3.1	4.37	5.6	6.2	Water	Water	C	C	CA/F	0.1-10.0
T438	Thidiazuron (95%)	220.2	51707-55-2	0.45	1.4	2.3	3.18	4.1	4.5	DMSO	N/A	RT	C	CA/F	0.001-0.05
T818	Triacontanol	438.8	593-50-0	0.22	0.7	1.1	1.59	2.1	2.3	EtOH/ DMSO	Water	C	F	F	0.01-10.0
T888	Thidiazuron	220.2	51707-55-2	0.45	1.4	2.3	3.18	4.1	4.5	DMSO	N/A	RT	C	CA/F	0.001-0.05
T841	*meta*-Topolin	241.5	N/A	0.41	1.2	2.1	2.89	3.7	4.1	KOH	Water	RT	F	F	0.01-5.0
T828	Trifluralin	335.3	1582-09-8	0.30	0.8	1.5	2.09	2.7	3.0	DMSO	Water	RT	C	F	—
T761	Trinexapac-Ethyl	252.3	95266-40-3	0.4	1.2	2.0	2.77	3.6	4.0	Water	Water	C	C	F	—
Z125	Zeatin	219.2	1637-39-4	0.45	1.4	2.3	3.19	4.1	4.6	KOH	Water	F	F	CA/F	0.01-5.0
Z860	Zeatin Solution, 1 mg/mL	219.2	13114-27-7	0.45	1.4	2.3	3.19	4.1	4.6	N/A	Water	F	F	CA/F	0.01-5.0
Z899	Zeatin Riboside	351.4	6025-53-2	0.28	0.9	1.4	1.99	2.6	2.9	KOH	Water	F	F	F	0.01-5.0
Z875	Zeatin Riboside Solution, 1 mg/mL	351.4	6025-53-2	0.28	0.9	1.4	1.99	2.6	2.9	N/A	Water	F	F	F	0.01-5.0

[1] mg/L to µM conversion example: 0.1 mg/L of ABA = 0.38 µM solution.

[2] Recommended concentration of KOH is 1 N.

Storage:
F = Freezer (-20 to 0 C)
C = Cooler/ Refrigerator (2-6 C)
RT = Room Temperature

Sterilize by:
CA = Co-autoclave with other media components
F = Filter Sterilize (Heat labile or no heat stability information available)
CA/F = Co-autoclave with media components, however, some loss of activity may occur

The above recommendations for storage and use are for informational purposes. End user assumes the responsibility for determining proper usage of the product.

SILVER THIOSULFATE SOLUTION PREPARATION

Prepare a 0.1 M Sodium Thiosulfate (STS) stock solution by dissolving 1.58 g of STS (Product No. S620) into 100 mL of water. Prepare a 0.1 M Silver Nitrate stock solution by dissolving 1.7 g of Silver Nitrate (Product No. S169) into 100 mL of water. Store the stock solution in the dark until needed to prepare the STS.

In general, the STS solution is prepared with a molar ratio between silver and thiosulfate of 1:4, respectively. Nearly all of the silver present in the solution is in the form of $[Ag(S_2O_3)_2]^{3-}$, the active complex for ethylene-effect inhibition.

Prepare a 0.02 M STS by slowly pouring 20 mL of 0.1 M silver nitrate stock solution into 80 mL of 0.1 M sodium thiosulfate stock solution. The STS can be stored in the refrigerator for up to a month. However, preparation of the STS just prior to use is recommended.

STERILIZER GLASS BEAD (Product No. S708)

*Phyto*Technology Laboratories offers a glass bead sterilizer for sterilization of instruments use in micropropagation. Product No. S708 has a chamber depth of 11.4 cm and chamber diameter of 6.3 cm. Small instruments such as scalpels and forceps are placed into the glass beads and are sterilized within 10-60 seconds. The instruments will cool down to working temperatures with 30-60 seconds. These units are very effective in killing microorganisms and their spores. **THESE UNITS ARE FOR STERILIZING METAL INSTRUMENTS ONLY!** To use these units simply follow the instructions below:

1. Fill the well with the glass beads from the bead packet. Using a funnel when pouring the beads will help prevent them from spilling.

2. Plug the sterilizer into an acceptable electrical outlet and turn the sterilizer power switch on. Allow approximately 30 minutes to heat up. The glass beads will reach a temperature of approximately 250° C. The unit is controlled by an electronic thermostat, which continuously cycles the heater on and off producing a uniform heating cycle.

3. Instruments should be wiped free of culture medium prior to insertion into the glass beads. This will ensure proper sterilization of the instruments and extend the life of the glass beads.

4. Insert clean, dry instruments into the glass beads for 10-15 seconds. Only the part of the instrument touching the glass beads will be sterilized. **DO NOT LEAVE THE INSTRUMENTS INSERTED IN THE GLASS BEADS FOR MORE THAN ONE MINUTE.**

5. The sterilizer can be left on all day without the outside portions of the unit becoming hot.

6. When you have finished with the sterilizer for the day, turn off the power switch.

REPLACING GLASS BEADS

With constant daily use the glass beads will last about one month. The glass beads should be cool before pouring them in a glass recycling or waste container. Refill the bead well with replacement glass beads (Product No. S638).

COMMONLY USED TISSUE CULTURE TECHNIQUES

Sterilization of Media

Plant tissue culture media are generally sterilized by autoclaving at 121° C and 1.05 kg/cm^2 (15 psi). The time required for sterilization depends upon the volume of medium in the vessel. The minimum times required for sterilization of different volumes of medium are listed below. It is advisable to dispense medium in small aliquots whenever possible as many media components are broken down on prolonged exposure to heat. There is evidence that medium exposed to temperatures in excess of 121° C may not properly gel or may result in poor cell growth.

MINIMUM AUTOCLAVING TIME FOR PLANT TISSUE CULTURE MEDIA

Volume of Medium Per Vessel (mL)	Minimum Autoclaving (min)[a]	Volume of Medium per Vessel (mL)	Minimum Autoclaving (min)[a]
25	20	500	35
50	25	1000	40
100	28	2000	48
250	31	4000	63

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

Several medium components are considered heat-labile and should not be autoclaved. Stock solutions of the heat-labile components are prepared and filter sterilized through a 0.22 µm filter into a sterile container. The filtered solution is aseptically added to the culture medium, which has been autoclaved and allowed to cool to approximately 35-45° C. The medium is then dispensed under sterile conditions. See the previous section "Stock Solutions: Sterilization of Media" for information regarding sterilization of heat-labile compounds commonly used in plant tissue culture. Experimentation with your system is recommended.

Surface Sterilizing Plant Material

To avoid bacterial and fungal growth, which is detrimental to culture growth, explants are surface sterilized before they are used to establish *in vitro* axenic cultures. The most common disinfectants are listed below with the concentration and exposure times that preserve the explants but at the same time destroy any microbial contamination.

PROCEDURES:

1. Wash explants in a mild detergent before treatment with the disinfecting solution. (Herbaceous material may not require this step.)
2. Rinse explants thoroughly under running tap water for 10-30 minutes.
3. Submerge explants into the disinfectant solution. Seal bottle and gently agitate.
4. Under sterile conditions, decant the solution and rinse explants several times with sterile distilled water.

STERILIZATION PROCEDURES MAY BE ENHANCED BY:

1. Placing the material in a 70% ethyl alcohol solution prior to treatment with another disinfectant solution. The use of a two step (two source) sterilization procedure has proven beneficial with certain species.
2. Using a wetting agent such as Tween 20 or 80 (*Phyto*Technology Product Nos. P720 or P738, respectively) can be added to the disinfectants to reduce surface tension and allow better surface contact.
3. Conducting the sterilization process under vacuum. This results in the removal of air bubbles and provides a more efficient sterilization process.

Orchid Seed Germination

Orchid seeds are very small and contain little or no food reserves. A single seed capsule may contain 1,500 to 3,000,000 seeds. Sowing the seed *in vitro* makes it possible to germinate immature seed (green pods). It is much easier to sterilize the green capsule than individual seed after the capsule has split open. Lucke (1971) indicated that orchid seed can be sterilized when the capsule is about two-thirds ripe. The following table provides the estimated normal ripening times of capsules for various orchid species (Lucke, 1971).

Orchid Genera	Time to Maturity (months)	Orchid Genera	Time to Maturity (months)	Orchid Genera	Time to Maturity (months)	Orchid Genera	Time to Maturity (months)
Bulbophyllum	3	Cymbidium	10	Laelia	9	Paphiopedilum	10
Calanthe	4	Cypripedum	3.5	Masdevallia	3.5	Phalaenopsis	6
Cattleya	11	Dendrobium	12	Miltonia	9	Stanhopea	7
Coelogyne	13	Epidendrum	3.5	Odontoglossum	7	Vanda	20

Green Capsule Sterilization:

1. Soak the capsule in a 100% bleach solution for 30 minutes.
2. Dip the capsule into 95% alcohol, and flame.
3. Under aseptic conditions open the capsule and scrape out the seed.
4. Carefully layer the seed over the surface of the culture medium.

Dry Seed Sterilization:

1. Collect seed and place in either a small flask or bottle or place in a shortened pipet, which has one end sealed with cotton. Seal the other end of the pipet with cotton once the seed has been placed in the pipet. Prepare a solution containing 5-10% commercial bleach containing a few drops of Tween 20 (Product No. P720).
2. Add the bleach solution to the flask or draw up the solution into the pipet. Swirl the flask containing the seed and bleach or repeatedly draw and aspirate the bleach solution in and out the pipet.
3. Sterilize the seed for a period of 5-10 minutes.
4. Remove the bleach solution and rinse the seed with sterile tissue culture grade water.
5. Transfer the seed from the pipet to sterile culture medium.

Replating Seedlings :

1. It may take anywhere from 1 to 9 months for the seed to begin to germinate. Approximately 30 to 60 days after germination begins, it may be necessary to transfer the seedlings to fresh media for continued growth.
2. Prepare an orchid maintenance/replate medium, such as P748 for epiphytic orchids or T849 for terrestrial orchids.
3. Under aseptic conditions, transfer the seedlings from the mother flask to the flask containing the fresh medium. You should place the seedling about ¼" apart on the medium.
4. Allow the seedlings to continue to grow and develop. Root formation generally begins when the plant has 2-3 leaves. Continue to transfer the seedlings to fresh media every 30-60 days, increasing the spacing between the plants with each transfer. When the flask is ready for transfer to a community pot in the greenhouse, most flasks will have 15 to 25 plants depending upon the species.
5. Transfer the plants into a community pot using a finely ground orchid mix.

PHYTOTECHNOLOGY LABORATORIES' CULTURE CONTAINER SELECTION GUIDE

Product Number	Product Description	Product Composition	Autoclavable	Sterile	Reusable	Dimensions in mm (round – d x h) (rectangular – l x w x h)	Approx. Surface Area of Medium (cm^2)	Number of Containers per T077 Tray	Approx. Number of Containers per ft^2	Recommended Closure
P700	*Phyto*Tech Culture Box™, Invertible Container	PS[1]	N	Y	N	100 x 100 x 105	64.	12	9	Included
C185	PC-120™ Culture Bottle, supplied w/ C070 *Phyto*Cap™	PC/PP[5]	Y	N	Y	64 x 64 x 120 42 mm opening	40	30	25	Included (C070)
C168	Culture Vessel, Clear-Con™	PS	N	Y	N	64 x 110	32.2	20	16	Included
C583	Culture Vessel, 9 oz. Glass Jar	Glass	Y	N	Y	73 x 87	41.8	20	16	Included (C566)
C597	Culture Vessel, 16 oz Glass Jar	Glass	Y	N	Y	89 x 95	62.2	12	9	Included (C579)
C590	Culture Vessel, 16 oz Glass Jar	Glass	Y	N	Y	77 x 135	46.5	12	10	Included (C566)
C031	Culture Vessel, 32 oz Glass Jar	Glass	Y	N	Y	85 x 174	35.3	20	16	C029 C566
C607	Culture Vessel, 32 oz Glass Jar	Glass	Y	N	Y	89 x 170	62.2	12	9	Included (C579)
C088	Culture Vessel, 8 oz – Round, Non-graduated	PP[2]	Y	N	Y	115 (rim) x 40 90 (base) x 40	63.6	9	7	Included
C209	*Phyto*Con™-8 Culture Vessel, 8 oz	PP	Y	N	Y	115 (rim) x 40 90 (base) x 40	63.6	9	7	Included
C093	Culture Vessel, 16 oz – Round, Non-graduated	PP	Y	N	Y	115 (rim) x 80 90 (base) x 80	63.6	9	7	Included
C215	*Phyto*Con™-16 Culture Vessel, 16 oz	PP	Y	N	Y	115 (rim) x 80 90 (base) x 80	63.6	9	7	Included
C221	*Phyto*Con™-32 Culture Vessel, 32 oz	PP	Y	N	Y	115 (rim) x 140 90 (base) x 140	63.6	9	7	Included
C532	Culture Vessel, 32 oz – Round, Non-Graduated	PP	Y	N	Y	145 (rim) x 80 122 (base) x 80	116.9	4	4	Included
C032	Culture Bottle, 32 oz, Square Milk Bottle	Glass	Y	N	Y	215 x 84 x 84	144	4	4	S982
E052	ECO 2 Culture Container – Rectangular, XXL Filters	CL - PP[3]	Y	N	Y	125 x 65 x 80	81.3	9	8.5	Included
E048	ECO 2 Culture Container – Rectangular, XL Filters	CL - PP	Y	N	Y	125 x 65 x 80	81.3	9	8.5	Included
E062	ECO 2 Culture Container – Round, XXL Filters	CL - PP	Y	N	Y	100 x 80	78.5	8	6	Included
E058	ECO 2 Culture Container – Round, XL Filters	CL - PP	Y	N	Y	100 x 80	78.5	8	6	Included
C906	Culture Vessel, Magenta GA7	PC/PP[5]	Y	N	Y	77 x 77 x 97	59.3	20	16	Included
C905	Culture Vessel, Magenta GA7-3	PC/PP	Y	N	Y	77 x 77 x 77	59.3	20	16	Included
C930	Culture Tubes, Round Bottom	Glass	Y	N	Y	25 x 150	4.9	—	—	C069, C909, C945

Continued on next page

Product Number	Product Description	Product Composition	Autoclavable	Sterile	Reusable	Dimensions in mm (round – d x h) (rectangular – l x w x h)	Approx. Surface Area of Medium (cm^2)	Number of Containers per T077 Tray	Number of Containers per ft^2	Recommended Closure
C935	Culture Tubes, Flat Bottom	Glass	Y	N	Y	25 x 95	4.9	—	—	C069, C909, C945
C944	Culture Vessel, Clear-Con™	PS	N	Y	N	68 x 68	32.2	20	16	Included
C915	Culture Bag, Star*Pac®, 2 Chamber	HDPE[4]	Y	N	N	6" x 9"	—	—	—	—
C916	Culture Bag, Star*Pac®, 5 Chamber	HDPE	Y	N	N	6" x 9"	—	—	—	—
C918	Culture Bag, Star*Pac®, Single Chamber	HDPE	Y	N	N	6" x 18"	—	—	—	—
C919	Culture Bag, Star*Pac®, Single Chamber	HDPE	Y	N	N	27" x 30"	—	—	—	—

1. PS = Polystyrene
2. PP = Polypropylene
3. CL-PP = Clarified Polypropylene
4. HDPE = High Density Polyethylene
5. PC/PP = Polycarbonate containers w/ Polypropylene lids

GLOSSARY

Adventitious---Developing from unusual points of origin, such as shoot or root tissues, from callus or embryos, from sources other than zygotes.
Agar---A polysaccharide powder derived from algae used to gel a medium. Agar is generally used at a concentration of 6-12 g/L.
Aseptic---Free of microorganisms.
Aseptic Technique---Procedures used to prevent the introduction of fungi, bacteria, viruses, mycoplasma or other microorganisms into cultures.
Autoclave---A machine capable of sterilizing wet or dry items with steam under pressure. Pressure cookers are a type of autoclave.
Auxin---A group of plant growth regulators that promotes callus growth, cell division, cell enlargement, adventitious buds, and lateral rooting. Indole-3-acetic (IAA) is a naturally occurring auxin. Examples of synthetic auxins included 2,4-Dichlorophenoxyacetic acid (2,4-D), Indole-3-Butyric acid (IBA), α-Naphthaleneacetic acid (NAA), and 4-Chlorophenoxyacetic acid (CPA).
Callus---An unorganized, proliferate mass of differentiated plant cells, a wound response.
Chemically Defined Medium---A nutritive solution for culturing cells in which each component is specifiable and ideally of known chemical structure.
Clone---Plants produced asexually from a single source plant.
Clonal Propagation---Asexual reproduction of plants that are considered to be genetically uniform and originated from a single individual or explant.
Contamination---Being infested with unwanted microorganisms such as bacteria or fungi.
Culture---A plant growing *in vitro*.
Cytokinin---A group of plant growth regulators that regulate growth and morphogenesis and stimulate cell division. Endogenous cytokinins: internal cytokinins that occur naturally, including timentin, zeatin and 6-γ,γ-dimethylallylaminopurine (2iP). Exogenous cytokinins: cytokinins that are applied, including natural ones and synthetic ones such as: 6-furfurylaminopurine (kinetin) and 6-benzylaminopurine (BA).
Differentiated---Cells that maintain, in culture, all or much of the specialized structure and function typical of the cell type *in vivo*. Modifications of new cells to form tissues or organs with a specific function.
Explant---Tissue taken from its original site and transferred to an artificial medium for growth or maintenance.
Gibberellins---A plant growth regulator that influences cell enlargement, promotes shoot elongation, and releases seeds and buds from dormancy. The most commonly used form of gibberellin is Gibberellic Acid (GA_3).
Horizontal laminar flow unit---An enclosed work area that has sterile air moving across it. The air moves with uniform velocity along parallel flow lines. Room air is pulled into the unit and forced through a HEPA (High Efficiency Particulate Air) filter, which removes particles 0.3 µm and larger.
Hormones---Naturally occurring plant growth regulators that affect plant growth and development in very low concentrations. (i.e., cytokinins, auxins, and gibberellins). They can be endogenous (internal, produced by the plant) or exogenous (exteranally applied).
Internode---The space between two nodes on a stem.
In vitro---To be grown in glass (Latin). Propagation of plants in a controlled, artificial environment using plastic or glass culture vessels, aseptic techniques, and a defined growing medium.
In vivo---To be grown naturally (Latin)

Media---Plural of medium
Medium---A nutritive solution, solid or liquid, for culturing cells.
Micropropagation---*In vitro* clonal propagation of plants from shoot tips or nodal explants, usually with an accelerated proliferation of shoots during subcultures.
Node---A part of the plant stem from which a leaf, shoot or flower originates.
Passage---The transfer or transplantation of cells or tissues with or without dilution or division, form one culture vessel to another.
Passage Number---The number of times the cells or tissues in culture have been subcultured or passaged.
Pathogen---A disease-causing organism.
Pathogenic---Capable of causing a disease.
Petiole---A leaf stalk; the portion of the plant that attaches the leaf blade to the node of the stem.
Plant Tissue Culture---The growth or maintenance of plant cells, tissues, organs or whole plants *in vitro.*
Regeneration---In plant cultures, a morphogenetic response to a stimulus that results in the products of organs, embryos, or whole plants.
Shoot Apical Meristem---Undifferentiated tissue, located within the shoot tip, generally appearing as a shiny dome-like structure, distal to the youngest leaf primordium and measuring less that 0.1 mm in length when excised.
Somaclonal Variation---Phenotypic variation, either genetic or epigenetic in origin, displayed among somaclones.
Somaclones---Plants derived from any form of cell culture involving the use of somatic plant cells.
Stage I---A step in *in vitro* propagation characterized by the establishment of an aseptic tissue culture of a plant.
Stage II---A step in *in vitro* propagation characterized by the rapid numerical increase of organs or other structures.
Stage III---A step in *in vitro* propagation characterized by preparation of propagules for successful transfer to soil, a process involving rooting of shoot cuttings, hardening of plants, and initiating the change from the heterotrophic to the autotrophic state.
Stage IV---A step in *in vitro* plant propagation characterized by the establishment in soil of a tissue culture derived plant, either after undergoing a Stage III pretransplant treatment, or in certain species, after the direct transfer of plants from Stage II into soil.
Sterile---(A) Without life. (B) Inability of an organism to produce functional gametes. (C) A culture that is free of viable microbes.
Sterile Techniques---The practice of working with cultures in an environment free from microorganisms.
Subculture---See "Passage". With plant cultures, this is the process by which the tissue or explant is first subdivided, then transferred into fresh culture medium.
Tissue Culture---The maintenance or growth of tissue, *in vitro*, in a way that may allow differentiation and preservation of their function.
Totipotency---A cell characteristic in which the potential for forming all the cell types in the adult organism are retained, thus theoretically able to reproduce the entire organism from one cell.
Undifferentiated---With plant cells, existing in a state of cell development characterized by isodiametric cell shape, very little or no vacuole, a large nucleus, and exemplified by cells comprising an apical meristem or embryo.

USEFUL GUIDES

Metric – US Conversion Table
(Approximate/ Average values)

US Measurement	Metric Weight	US Volume	Metric Volume
¼ tsp	0.4 g	1 tsp	5 mL
1¼ tsp	2 g	1 fl oz	30 mL
3 tsp (1Tbs)	5 g	1 pint (16 fl oz)	473 mL
1 oz	28 g	34 fl oz	1 L
1 lb	454 g	1 gal	3.8 L

Flask/Container — Stopper Chart

Flask/ Container		Stopper	
Size	Product No.	Size	Product No.
125 mL	F934	No. 6½	S981
250 mL	F979	No. 8	S983
300 mL	F938	No. 8	S983
500 mL	F980	No. 10	S984
Culture Bottle	C032	No. 7	S982

Tris Buffer Preparation Guide
Dissolve Tris HCl & Base in distilled/ deionized water.

g/L for 50 mM Solution		pH at Temperature	
Tris HCl	Tris Base	5° C	25° C
7.02	0.67	7.76	7.20
6.85	0.80	7.89	7.30
6.61	0.97	7.97	7.40
6.35	1.18	8.07	7.50
6.06	1.39	8.18	7.60
5.72	1.66	8.26	7.70
5.32	1.97	8.37	7.80
4.88	2.3	8.48	7.90
4.44	2.65	8.58	8.0
4.02	2.97	8.68	8.10
3.54	3.34	8.78	8.20
3.07	3.70	8.88	8.30
2.64	4.03	8.98	8.40
2.21	4.36	9.09	8.50
1.83	4.65	9.18	8.60
1.50	4.90	9.28	8.70
1.23	5.13	9.36	8.80
0.96	5.32	9.47	8.90
0.76	5.47	9.56	9.0

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS

All components expressed in mg/L	Anderson Basal Salt Mixture	Banana AGS Medium	BY-2 Medium	Blaydes Modified Basal Medium	Chu N6 Basal Medium w/ Vitamins	Cape Sundew/ Venus Fly Trap Multip. Medium	Carrot Callus Initiation medium	Cape Sundew/ Venus Fly Trap Pretransplant Medium	Carrot Shoot Development medium
COMPONENT	**A267**	**B144**	**B468**	**B514**	**C167**	**C206**	**C212**	**C216**	**C222**
Ammonium Nitrate	400.0	1,650.0	1,650.0	1,000.0		400.0		825.0	
Ammonium Sulfate					463.0		134.0		134.0
Boric Acid	6.20	6.20	6.20	1.60	1.60	6.20	3.00	3.10	3.00
Calcium Chloride, Anhydrous	332.200	333.000	332.200		125.330	332.200	113.240	166.500	113.240
Calcium Nitrate				241.100					
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250			0.0250	0.0250	0.0125	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250			0.0250	0.0250	0.0125	0.0250
Na_2 EDTA·$2H_2O$	74.50		37.26	74.50	37.25	74.50	37.26		37.26
Ferric Sodium EDTA		36.700						18.350	
Ferrous Sulfate·$7H_2O$	55.70		27.8	55.70	27.85	55.70	27.80		27.80
Magnesium Sulfate, Anhydrous	180.700	181.000	180.700	17.100	90.370	180.700	122.090	90.500	122.090
Manganese Sulfate·H_2O	16.900	16.900	16.900	4.400	3.300	16.900	10.000	8.450	10.000
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250			0.250	0.250	0.125	0.250
Potassium Chloride				100.000					
Potassium Iodide	0.300	0.830	0.830	0.800	0.800		0.750	0.415	0.750
Potassium Nitrate	480.00	1,900.00	1,900.00	65.00	2,830.00	480.00	2,500.00	950.00	2,500.00
Potassium Phosphate, Monobasic, Anhydrous		170.0	370.0	300.0	400.0			85.0	
Sodium Phosphate Monobasic·H_2O	330.6	295.0				380.0	150.0		150.0
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	1.500	1.500	8.600	2.000	4.300	2.000
Adenine Hemisulfate·$2H_2O$						80.000			
2,4-Dichlorophenoxyacetic Acid			0.200				1.000		
6-γ,γ-Dimethylallylaminopurine (2IP)		10.000				1.000			
Glycine				2.000	2.000				
Indole-3-acetic Acid		1.000							
Kinetin									0.200
myo-Inositol		100.0	100.0			100.0	100.0	50.0	100.0
Nicotinic Acid					0.50		1.00		1.00
Pyridoxine·HCl					0.50		1.00		1.00
Sucrose			30,000.0	30,000.0					
Thiamine·HCl		0.40		0.10	1.00	0.40	10.00	0.20	10.00
Grams of powder to prepare 1 liter	1.890	4.710	34.630	31.860	3.990	2.120	3.210	2.200	3.210
pH±0.5 at RT	3.75	4.75	NS	NS	4.00	3.50	4.00	5.00	4.00

NS = No Specification Established

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Chee & Pool C2d Vitis Medium	Chu N6 Basal Salt Mixture	DCR Basal Salt Mixture	DKW Basal Salt Mixture w/ Sucrose	DKW Basal Salt Mixture	DKW Basal Salt Mixture W/ 10.0 g/L Sucrose	EXS IIIa™ Basal Medium	EXS III™ Basal Medium	Economou & Read Basal Medium
COMPONENT	**C287**	**C416**	**D146**	**D189**	**D190**	**D191**	**E333**	**E337**	**E575**
Ammonium Nitrate	1,650.0		400.0	1,416.0	1,416.0	1,416.0	Proprietary Formulation	Proprietary Formulation	400.0
Ammonium Phosphate, Monobasic									
Ammonium Sulfate		463.0							132.0
Boric Acid	6.20	1.60	6.20	4.80	4.80	4.80			6.20
Calcium Chloride, Anhydrous		125.330	64.140	112.500	112.500	112.500			332.200
Calcium Nitrate	492.300		386.310	1,367.000	1,367.000	1,367.000			
Cobalt Chloride·$6H_2O$	0.0250		0.0250						0.0250
Cupric Sulfate·$5H_2O$	0.0250		0.2500	0.2500	0.2500	0.2500			0.0250
Na_2 EDTA·$2H_2O$	37.30	37.25	37.30	45.40	45.40	45.40			
Ferrous Sulfate·$7H_2O$	27.80	27.85	27.80	33.80	33.80	33.80			
Iron Chelate, Sequestrene® 330									56.000
Magnesium Sulfate, Anhydrous	180.600	90.370	180.700	361.490	361.490	361.490			180.700
Manganese Sulfate·H_2O	0.845	3.300	22.300	33.500	33.500	33.500			16.900
Sodium Molybdate(VI)·$2H_2O$	0.250		0.250	0.390	0.390	0.390			0.250
Nickel Chloride·$6H_2O$			0.025						
Nickel Sulfate·$6H_2O$				0.005	0.005	0.005			
Potassium Iodide		0.800	0.830						
Potassium Nitrate	1,900.00	2,830.00	340.00						202.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	400.0	170.0	265.0	265.0	265.0			408.0
Potassium Sulfate				1,559.000	1,559.000	1,559.000			
Zinc Nitrate·$6H_2O$				17.000	17.000	17.000			
Zinc Sulfate·$7H_2O$	8.600	1.500	8.600						8.600
myo-Inositol	10.0								100.0
Nicotinic Acid	1.00								
Pyridoxine·HCl	1.00								
Sucrose				30,000.0		10,000.0			
Thiamine·HCl	1.00								0.40
Grams of powder to prepare 1 liter	4.490	4.000	1.640	35.220	5.220	15.220	3.950	3.870	1.840
pH±0.5 at RT	4.00	4.25	4.00	4.00	4.00	4.00	4.00	4.75	NS

NS = No Specification Established

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Gamborg (PRL-4-DM) Long Medium	Gresshoff & Doy Basal Slat Mixture	Gamborg B-5 Basal Medium	Gamborg Basal Salt Mixture	Hoagland Modified Basal Salt Mixture	Heller Basal Salt Mixture	Heller/ White Modified Basal Salt Mixture
COMPONENT	**G359**	**G371**	**G398**	**G768**	**H353**	**H393**	**H396**
Aluminum Chloride·6H_2O						0.054	0.030
Ammonium Nitrate		1,000.0					
Ammonium Phosphate, Monobasic					115.03		
Ammonium Sulfate	200.0		134.0	134.0			
Boric Acid	3.00	0.30	3.00	3.00	2.86	1.00	1.24
Calcium Chloride, Anhydrous	113.240		113.240	113.240		56.700	
Calcium Nitrate		241.200			656.400		300.000
Cobalt Chloride·6H_2O	0.2500	0.0250	0.0250	0.0250			
Cupric Sulfate·5H_2O	0.2500	0.0250	0.0250	0.0250	0.0800	0.0300	0.0300
Na_2 EDTA·2H_2O	186.00	37.25	37.26	37.26	3.53		
Ferric Chloride						1.000	
Ferric Sulfate·XH_2O							25.0
Ferrous Sulfate·7H_2O	139.00	27.85	27.80	27.80	2.50		
Magnesium Sulfate, Anhydrous	122.090	17.099	122.090	122.090	240.760	122.100	351.630
Manganese Chloride·4H_2O					1.810		
Manganese Sulfate·H_2O	132.000	1.000	10.000	10.000		0.076	0.010
Sodium Molybdate(VI)·2H_2O	0.250	0.025	0.250	0.250			
Molybdenum Trioxide					0.016		
Nickel Chloride·6H_2O						0.030	0.030
Potassium Chloride	300.000	65.000				750.000	65.000
Potassium Iodide	0.750	0.800	0.750	0.750		0.010	0.010
Potassium Nitrate	1,000.00	1,000.00	2,500.00	2,500.00	606.60		80.00
Potassium Phosphate, Monobasic, Anhydrous		300.0					
Sodium Nitrate						600.000	
Sodium Phosphate Monobasic·H_2O	90.0		150.0	150.0		108.8	16.5
Sodium Sulfate							200.000
Zinc Sulfate·7H_2O	3.000	0.300	2.000	2.000	0.220	1.000	1.000
p-Aminobenzoic Acid	0.200						
L-Arginine (Free Base)	40.000						
L-Ascorbic Acid	0.400						
Asparagine	40.000						
D-Biotin	0.000	0.200					
Calcium Pantothenate	0.400						
Choline Chloride	0.200						
Folic Acid	0.015						
L-Glutamine	60.000						
Glycine	20.000	4.000					
myo-Inositol	100.0	10.0	100.0				
Methionine	30.000						
Nicotinic Acid	0.50	0.10	1.00				
L-Phenylalanine	20.00						
Pyridoxine·HCI	0.50	0.10	1.00				
Riboflavin, Vit. B_2	0.015						
Thiamine·HCI	0.50	1.00	10.00				
L-Tryptophan	40.0						
Grams of powder to prepare 1 liter	3.300	2.710	3.210	3.100	1.630	1.640	1.040
pH±0.5 at RT	3.75	4.00	4.00	4.00	4.75	4.90	4.70

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Hosta Initiation/ Multiplication Medium	Hosta Multiplication Medium	Hosta Rooting Medium	Kao & Michayluk Basal Salt Mixture	Kao & Michayluk Modified Basal Medium	Lloyd and McCown Woody Plant Basal Salt Mix.	Lloyd and McCown Woody Plant Micronut. Mixture	Lloyd and McCown Woody Plant Medium
COMPONENT	**H435**	**H436**	**H437**	**K413**	**K427**	**L154**	**L444**	**L449**
Ammonium Nitrate	1,650.0	1,650.0	1,650.0	600.0	600.0	400.0		400.0
Boric Acid	6.20	6.20	6.20	3.00	3.00	6.20	6.20	6.20
Calcium Chloride, Anhydrous	332.200	332.200	332.200	453.000	453.000	72.500	72.500	72.500
Calcium Nitrate						386.000		386.000
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250			
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.2500	0.2500	0.2500
Na_2 EDTA·$2H_2O$	37.26	37.26	37.26	37.26	37.26	37.30	37.30	37.30
Ferrous Sulfate·$7H_2O$	27.80	27.80	27.80	27.85	27.85	27.90	27.85	27.90
Magnesium Sulfate, Anhydrous	180.700	180.700	180.700	146.550	146.550	180.700	180.700	180.700
Manganese Sulfate·H_2O	16.900	16.900	16.900	10.000	10.000	22.300	22.300	22.300
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Potassium Chloride				300.000	300.000			
Potassium Iodide	0.830	0.830	0.830	0.750	0.750			
Potassium Nitrate	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00			
Potassium Phosphate, Monobasic, Anhydrous	300.0	300.0	300.0	170.0	170.0	170.0	170.0	170.0
Potassium Sulfate						990.000		990.000
Sodium Phosphate Monobasic·H_2O	170.0	170.0	170.0					
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	2.000	2.000	8.600	8.600	8.600
Adenine Hemisulfate·$2H_2O$	160.000	160.000						
Agar	8,000.0	8,000.0	8,000.0					
p-Aminobenzoic Acid					0.020			
L-Ascorbic Acid					2.000			
6-Benzylaminopurine	2.000	0.100	0.100					
D-Biotin					0.010			
Calcium Pantothenate					1.000			
Casein, Enzymatic Hydrolysate	500.000	500.000	500.000					
Cholecalciferol, Vit. D_3					0.010			
Choline Chloride					1.000			
Citric Acid, Anhydrous					40.000			
Cyanocobalamin, Vit B_{12}					0.020			
Folic Acid					0.400			
Fumaric Acid					40.000			
Glycine	2.000	2.000						2.000
L-Malic Acid					40.000			
myo-Inositol	100.0	100.0	100.0		100.0			100.0
α-Naphthaleneacetic Acid	0.500	0.500	0.500					
Niacinamide					1.00			
Nicotinic Acid								0.50
Pyridoxine·HCl					1.00			0.50
Pyruvic Acid, Potassium Salt					20.00			
Retinol, Vit. A					0.010			
Riboflavin, Vit. B_2					0.200			
Sucrose	30,000.0	30,000.0	30,000.0					
Thiamine·HCl	0.40	0.40	0.40		1.00			1.00
Grams of powder to prepare 1 liter	43.400	43.390	43.230	3.650	3.900	2.300	0.530	2.410
pH±0.5 at RT	4.30	4.30	4.30	4.00	3.50	4.00	4.00	4.00

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Linsmaier & Skoog Basal Medium, Buffered & pH Adjusted	Linsmaier & Skoog Basal Medium	Linsmaier & Skoog Basal Med. w/ 30 g/L Sucrose, pH Adjusted & Buffered	Linsamaier and Skoog Basal Medium (MSMO)	Litvay Basal Salt Mixture	Linsamaier and Skoog Basal Medium (MSMO)
COMPONENT	**L452**	**L467**	**L473**	**L477**	**L546**	**L689**
Ammonium Nitrate	1,650.0	1,650.0	1,650.0	1,650.0	1,650.0	1,650.0
Boric Acid	6.20	6.20	6.20	6.20	31.00	6.20
Calcium Chloride, Anhydrous	332.200	332.200	332.200	332.200	16.610	332.200
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250	0.1250	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.5000	0.0250
Na_2 EDTA·$2H_2O$	37.26	37.26	37.26	37.26		37.26
Ferric Sodium EDTA					36.700	
Ferrous Sulfate·$7H_2O$	27.80	27.80	27.80	27.80		27.80
Magnesium Sulfate, Anhydrous	180.700	180.700	180.700	180.700	903.380	180.700
Manganese Sulfate·H_2O	16.900	16.900	16.900	16.900	21.000	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	1.250	0.250
Potassium Hydroxide	100.000		100.000	100.000		
Potassium Iodide	0.830	0.830	0.830	0.830	4.150	0.830
Potassium Nitrate	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0	170.0	170.0	340.0	170.0
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	8.600	43.000	8.600
Agar	7,000.0	7,000.0				
MES (Free Acid)	1,000.0		1,000.0	1,000.0		
myo-Inositol	100.0	100.0	100.0	100.0		100.0
Sucrose	30,000.0	30,000.0	30,000.0			
Thiamine·HCl	0.40	0.40	0.40	0.40		0.40
Grams of powder to prepare 1 liter	42.530	41.430	35.530	5.530	4.950	4.430
pH±0.5 at RT	5.75	4.75	5.75	5.75	4.75	4.00

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	MS Modified Basal Salt Mixture (1/2x Micros & Macros)	MS Modified Basal Salt Mixture	MS Modified Medium w/ BA & NAA	MS Modified Basal Medium w/ Gamborg Vitamins	MS Modified Basal Salt Mixture (No N, P, or K)	MG Mediuim (Modified MS/ Gamborg Medium)	Musa (Banana) Multiplication Medium
COMPONENT	**M153**	**M290**	**M401**	**M404**	**M407**	**M419**	**M462**
Ammonium Nitrate	825.0	825.0	1,650.0	1,650.0			1,650.0
Ammonium Sulfate						33.5	
Boric Acid	3.10	6.20	6.20	6.20	6.20	2.30	6.20
Calcium Chloride, Anhydrous	166.100	166.100	332.200	332.200	332.200	111.360	332.200
Cobalt Chloride·$6H_2O$	0.0125	0.0250	0.0250	0.0250	0.0250	0.0125	0.0250
Cupric Sulfate·$5H_2O$	0.0125	0.0250	0.0250	0.0250	0.0250	0.0125	0.0250
Na_2 EDTA·$2H_2O$	18.63	37.26	37.26	37.26	37.26	18.64	37.25
Ferrous Sulfate·$7H_2O$	13.90	27.80	27.80	27.80	27.80	13.90	27.85
Magnesium Sulfate, Anhydrous	90.350	180.700	180.700	180.700	180.700	75.700	180.740
Manganese Sulfate·H_2O	8.450	16.900	16.900	16.900	16.900	6.700	16.900
Sodium Molybdate(VI)·$2H_2O$	0.125	0.250	0.250	0.250	0.250	0.125	0.250
Potassium Iodide	0.415	0.830	0.830	0.830	0.830	0.400	0.830
Potassium Nitrate	950.00	950.00	1,900.00	1,900.00		1,100.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	85.0	170.0	170.0	170.0		42.5	170.0
Sodium Nitrate						437.800	
Sodium Phosphate Monobasic·H_2O						32.6	
Zinc Sulfate·$7H_2O$	4.300	8.600	8.600	8.600	8.600	2.700	8.600
L-Ascorbic Acid							20.000
6-Benzylaminopurine			1.000				4.500
Gellan Gum (CultureGel™)							2,000.000
Glycine			2.000				2.000
Indole-3-acetic Acid							0.175
myo-Inositol			100.0	100.0			
α-Naphthaleneacetic Acid			0.100				
Nicotinic Acid			0.50	1.00			0.50
Pyridoxine·HCl			0.50	1.00			0.50
Sucrose			30,000.0				30,000.0
Thiamine·HCl			0.40	10.00			0.40
Grams of powder to prepare 1 liter	2.170	2.390	34.440	4.440	0.610	1.880	36.360
pH±0.5 at RT	4.25	4.00	4.00	4.00	4.00	4.30	4.25

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Murashige Modified Shoot Multiplication Basal Medium	MS Modified Basal Salt Mixture w/ FeNaEDTA	MS Macronutrient Salt Base	Murashige & Skoog Basal Salt Mixture - Finer & Nagasawa Modification	Murashige Modified Fern Multiplication Basal Medium	Murashige Modified Gerbera Multiplication Basal Medium	Murashige Modified Gerbera Pretransplant Basal Medium
COMPONENT	**M491**	**M499**	**M502**	**M504**	**M508**	**M509**	**M510**
Ammonium Nitrate	1,650.0	1,650.0	1,650.0	825.0	1,650.0	1,650.0	1,650.0
Boric Acid	6.20	6.20		6.20	6.20	6.20	6.20
Calcium Chloride, Anhydrous	333.000	333.000	332.200	332.200	333.000	333.000	333.000
Cobalt Chloride·$6H_2O$	0.0250	0.0250		0.0250	0.0250	0.0250	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250		0.0250	0.0250	0.0250	0.0250
Ferric Sodium EDTA	36.700	36.700		36.700	36.700	36.700	36.700
Magnesium Sulfate, Anhydrous	181.000	181.000	180.700	180.540	181.000	181.000	181.000
Manganese Sulfate·H_2O	16.900	16.900		16.900	16.900	16.900	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250		0.250	0.250	0.250	0.250
Potassium Iodide	0.830	0.830		0.830	0.830	0.830	0.830
Potassium Nitrate	1,900.00	1,900.00	1,900.00	3,030.00	1,900.00	1,900.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0	170.0	170.0	170.0	170.0	170.0
Sodium Phosphate Monobasic·H_2O	170.0				255.0	85.0	85.0
Zinc Sulfate·$7H_2O$	8.600	8.600		8.600	8.600	8.600	8.600
Adenine Hemisulfate·$2H_2O$	80.000					80.000	
6-γ,γ-Dimethylallylaminopurine (2IP)	30.000						
Indole-3-acetic Acid	0.300					0.500	10.000
Kinetin					2.000	10.000	
myo-Inositol	100.0				100.0	100.0	100.0
α-Naphthaleneacetic Acid					0.100		
Nicotinic Acid						10.00	10.00
Pyridoxine·HCl						1.00	1.00
Thiamine·HCl	0.40				0.40	30.00	30.00
L-Tyrosine, Free Base						100.0	100.0
Grams of powder to prepare 1 liter	4.680	4.300	4.230	4.610	4.660	4.720	4.640
pH±0.5 at RT	4.90	4.75	4.75	NS	4.75	4.00	4.25

NS = No Specification Established

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Murashige Modified Kalanchoe Multplication Basal Medium	Murashige Modified Kalanchoe Pre-transplant Basal Medium	Murashige Modified Lily Multiplication Basal Medium	MS Modified BC Potato Basal Medium	Murashige Modified African Violet/ Gloxinia Multiplication Basal Medium	Murashige Modified African Violet/ Gloxinia Pretransplant Basal Medium	Murashige & Skoog Basal Medium
COMPONENT	**M511**	**M512**	**M513**	**M516**	**M517**	**M518**	**M519**
Ammonium Nitrate	1,650.0	1,650.0	1,650.0	1,650.0	1,650.0	1,650.0	1,650.0
Boric Acid	6.20	6.20	6.20	6.20	6.20	6.20	6.20
Calcium Chloride, Anhydrous	333.000	333.000	333.000	333.000	333.000	333.000	332.200
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250
Na_2 EDTA·$2H_2O$							37.26
Ferric Sodium EDTA	36.700	36.700	36.700	36.700	36.700	36.700	
Ferrous Sulfate·$7H_2O$							27.80
Magnesium Sulfate, Anhydrous	181.000	181.000	181.000	181.000	181.000	181.000	180.700
Manganese Sulfate·H_2O	16.900	16.900	16.900	16.900	16.900	16.900	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Potassium Iodide	0.830	0.830	0.830	0.830	0.830	0.830	0.830
Potassium Nitrate	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0	170.0	170.0	170.0	170.0	170.0
Sodium Phosphate Monobasic·H_2O					170.0		
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	8.600	8.600	8.600	8.600
Adenine Hemisulfate·$2H_2O$					80.000		
6-γ,γ-Dimethylallylaminopurine (2IP)	3.000						
Glycine				2.000			2.000
Indole-3-acetic Acid		3.000			2.000	1.000	
Kinetin				0.040	2.000		
myo-Inositol	100.0	100.0	100.0	100.0	100.0	100.0	100.0
α-Naphthaleneacetic Acid			0.030				
Nicotinic Acid				0.50			0.50
Pyridoxine·HCl				0.50			0.50
Thiamine·HCl	0.40	0.40	0.40	0.40	0.40	0.40	0.10
Grams of powder to prepare 1 liter	4.410	4.410	4.400	4.410	4.660	4.400	4.430
pH±0.5 at RT	4.75	4.75	4.75	4.75	4.00	475.00	3.90

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Murashige & Skoog (MS) Basal Salt Mixture	Murashige Modified Multiplication Basal Medium w/ Kinetin & IAA	MS Micronutrient Stock Solution (10x)	MS Modified Basal Medium Buffered w/ MES	MS Modified Basal Salt Mixture (No Nitrogen)	MS Modified Basal Medium	Murashige Modified Multiplication Basal Medium w/ 2iP
COMPONENT	**M524**	**M527**	**M529**	**M530**	**M531**	**M535**	**M536**
Ammonium Nitrate	1,650.0	1,650.0		1,650.0		1,650.0	1,650.0
Boric Acid	6.20	6.20	62.00	6.20	6.20	6.20	6.20
Calcium Chloride, Anhydrous	332.200	333.000		332.200	332.200	332.200	332.200
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.2500	0.0250	0.0250	0.0250	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.2500	0.0250	0.0250	0.0250	0.0250
Na_2 EDTA·$2H_2O$	37.26		373.00	37.26	37.26	37.26	37.26
Ferric Sodium EDTA		36.700					
Ferrous Sulfate·$7H_2O$	27.80		278.00	27.80	27.80	27.80	27.80
Magnesium Sulfate, Anhydrous	180.700	181.000		180.700	180.700	180.700	180.700
Manganese Sulfate·H_2O	16.900	16.900	169.000	16.900	16.900	16.900	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	2.500	0.250	0.250	0.250	0.250
Potassium Iodide	0.830	0.830	8.300	0.830	0.830	0.830	0.830
Potassium Nitrate	1,900.00	1,900.00		1,900.00		1,900.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0		170.0	170.0	170.0	170.0
Sodium Phosphate Monobasic·H_2O							170.0
Zinc Sulfate·$7H_2O$	8.600	8.600	86.000	8.600	8.600	8.600	8.600
Adenine Hemisulfate·$2H_2O$						80.000	80.000
Glycine				2.000			2.000
Indole-3-acetic Acid		0.300					
Kinetin		1.000					
MES (Free Acid)				1,000.0			
myo-Inositol		100.0		100.0		100.0	100.0
Nicotinic Acid				0.50			0.50
Pyridoxine·HCl				0.50			0.50
Thiamine·HCl		0.40		0.10		0.40	0.40
Grams of powder to prepare 1 liter	4.330	4.410	NA	5.430	0.780	4.510	4.680
pH±0.5 at RT	4.00	4.75	3.00	NS	4.00	3.75	4.75

NS = No Specification Established

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	MS Modified Basal Medium (w/out KH2PO4)	MS Modified Medium (Arabidopsis)	MS Micronutrient Salt Base	MS Modified Multiplication Medium w/ Kinetin	MS Modified Basal Salt Mixture (1/2x Nitrates)	MS Modified Basal Salt Mixture (No NH_4NO_3)	MS Basal Salt Concentrate (20x)	MS Macronutrient Stock Solution (10x)
COMPONENT	**M541**	**M550**	**M554**	**M555**	**M561**	**M571**	**M576**	**M654**
Ammonium Nitrate	1,650.0	1,650.0		1,650.0	825.0		33,000.0	16,500.0
Boric Acid	6.20	6.20	6.20	6.20	6.20	6.20	124.00	
Calcium Chloride, Anhydrous	332.200	332.200		332.200	332.200	332.200	6,644.000	3,322.000
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.5000	
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.5000	
Na_2 EDTA·$2H_2O$		37.26	37.26	37.26	37.26	37.26	745.20	
Ferric Sodium EDTA	36.700							
Ferrous Sulfate·$7H_2O$		27.80	27.80	27.80	27.80	27.80	556.00	
Magnesium Sulfate, Anhydrous	180.700	180.700		180.700	180.700	180.700	3,614.000	1,810.000
Manganese Sulfate·H_2O	16.900	16.900	16.900	16.900	16.900	16.900	338.000	
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	0.250	0.250	5.000	
Potassium Iodide	0.830	0.830	0.830	0.830	0.830	0.830	16.600	
Potassium Nitrate	1,900.00	1,900.00		1,900.00	950.00	1,900.00	38,000.00	19,000.00
Potassium Phosphate, Monobasic, Anhydrous		170.0		170.0	170.0	170.0	3,400.0	1,700.0
Sodium Phosphate Monobasic·H_2O	300.0			148.0				
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	8.600	8.600	8.600	172.000	
Adenine Hemisulfate·$2H_2O$	150.000			80.000				
Casein, Enzymatic Hydrolysate	1,000.000							
2,4-Dichlorophenoxyacetic Acid		2.000						
Glycine	2.000							
Kinetin		0.050		1.000				
myo-Inositol	100.0	100.0		100.0				
α-Naphthaleneacetic Acid				0.100				
Nicotinic Acid	5.00	1.00						
Pyridoxine·HCl	1.00	1.00						
Sucrose		20,000.0		30,000.0				
Thiamine·HCl	0.50	10.00		0.40				
Grams of powder to prepare 1 liter	5.690	24.440	0.100	34.660	2.560	2.680	N/A	N/A
pH±0.5 at RT	4.75	3.75	4.00	3.75	4.00	4.00	3.25	4.25

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	MS Modified Basal Medium w/ 2iP	MS Modified Medium w/ 2iP & IAA	NLN Medium	NB Basal Medium	Nitsch & Nitsch Basal Salt Mixture	Nitsch & Nitsch Basal Medium	Parker-Thompson Fern Basal Salt Mixture	Quoirin & Lepoivre Basal Salt Mixture	Rose Initiation Medium
COMPONENT	**M701**	**M702**	**N479**	**N492**	**N613**	**N616**	**P713**	**Q673**	**R756**
Ammonium Molybdate							0.037		
Ammonium Nitrate	1,650.0	1,650.0			720.0	720.0	125.0	400.0	1,650.0
Ammonium Sulfate				463.0					
Boric Acid	6.20	6.20	10.00	3.00	10.00	10.00	1.86	6.20	6.20
Calcium Chloride, Anhydrous	333.000	332.200		125.330	166.000	166.000	19.628		333.000
Calcium Nitrate			345.000					833.770	
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250				0.0250	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.3700	0.0250	0.0250
Na_2 EDTA·$2H_2O$		37.26		37.26	37.26	37.26	37.30	37.30	
Ferric Sodium EDTA	36.700		36.700						36.700
Ferrous Sulfate·$7H_2O$		27.80		27.80	27.80	27.80	27.80	27.80	
Magnesium Sulfate, Anhydrous	181.000	180.700	61.000	90.370	90.372	90.372	58.565	175.790	181.000
Manganese Sulfate·H_2O	16.900	16.900	18.950	10.000	18.900	18.900	0.250	0.760	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	0.250	0.250		0.250	0.250
Potassium Iodide	0.830	0.830		0.750				0.080	0.830
Potassium Nitrate	1,900.00	1,900.00	125.00	2,830.00	950.00	950.00		1,800.00	1,900.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0	125.0	400.0	68.0	68.0	500.0	270.0	170.0
Sodium Phosphate Monobasic·H_2O		148.0							
Zinc Sulfate·$7H_2O$	8.600	8.600	10.000	2.000	10.000	10.000	0.520	8.600	8.600
Adenine Hemisulfate·$2H_2O$	30.000	80.000							
L-Ascorbic Acid									50.000
6-Benzylaminopurine									2.000
D-Biotin			0.050			0.050			
Citric Acid, Anhydrous									50.000
6-γ,γ-Dimethylallylaminopurine (2IP)	10.000	30.000							
Folic Acid			0.500			0.500			
L-Glutamine			800.000						
Glutathione (Reduced), Sodium Salt			30.000						
Glycine			2.000			2.000			2.000
Indole-3-acetic Acid	1.000	0.300							0.300
myo-Inositol	100.0	100.0	100.0	100.0		100.0			100.0
Nicotinic Acid			5.00	1.00		5.00			0.50
Pyridoxine·HCl			0.50	1.00		0.50			0.50
L-Serine			100.0						
Sucrose		30,000.0							
Thiamine·HCl	0.40	0.40	0.50	10.00		0.50			0.40
Grams of powder to prepare 1 liter	4.440	34.690	1.770	4.100	2.100	2.210	0.770	3.560	4.510
pH±0.5 at RT	4.25	4.00	4.75	4.00	4.00	4.80	4.00	4.00	3.50

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Rose Multiplication Medium	Rose Rooting Medium	Schenk & Hildebrandt Modified Basal Salts	Schenk & Hildebrandt Modified Basal Salts	Schenk & Hildebrandt Basal Salts w/ Sucrose	Schenk & Hildebrandt Modified Basal Medium	Schenk & Hildebrandt Basal Salts	TM4G Basal Salts
COMPONENT	**R757**	**R758**	**S806**	**S808**	**S811**	**S813**	**S816**	**T853**
Ammonium Nitrate	1,650.0	412.5						320.0
Ammonium Phosphate, Monobasic			300.00	150.00	300.00	300.00	300.00	230.00
Ammonium Sulfate								130.0
Boric Acid	6.20	1.55	5.00	2.50	5.00	5.00	5.00	6.20
Calcium Chloride, Anhydrous	333.000	83.250		75.500	151.000	151.000	151.000	113.250
Cobalt Chloride·$6H_2O$	0.0250	0.0063	0.1000	0.0500	0.1000	0.1000	0.1000	0.0250
Cupric Sulfate·$5H_2O$	0.0250	0.0063	0.2000	0.1000	0.2000	0.2000	0.2000	0.0250
Na_2 EDTA·$2H_2O$			20.00	10.00	20.00	20.00	20.00	18.65
Ferric Sodium EDTA	36.700	9.175						
Ferrous Sulfate·$7H_2O$			15.00	7.50	15.00	15.00	15.00	13.90
Magnesium Sulfate, Anhydrous	181.000	45.250	195.400	97.700	195.400	195.400	195.400	122.124
Manganese Sulfate·H_2O	16.900	4.225	10.000	5.000	10.000	10.000	10.000	16.900
Sodium Molybdate(VI)·$2H_2O$	0.250	0.063	0.100	0.050	0.100	0.100	0.100	0.250
Potassium Iodide	0.830	0.208	1.000	0.500	1.000	1.000	1.000	0.830
Potassium Nitrate	1,900.0	475.0	2,500.0	1,250.0	2,500.0	2,500.0	2,500.0	1,900.0
Potassium Phosphate, Monobasic, Anhydrous	170.0	42.5						
Zinc Sulfate·$7H_2O$	8.600	2.150	1.000	0.500	1.000	1.000	1.000	9.200
L-Ascorbic Acid	50.000							
6-Benzylaminopurine	3.000							
Citric Acid, Anhydrous	50.000							
Glycine	2.000	2.000						
Indole-3-acetic Acid	0.300							
myo-Inositol	100.0	100.0		500.0		1,000.0		
α-Naphthaleneacetic Acid		0.030						
Nicotinic Acid	0.50	0.50		2.50		5.00		
Pyridoxine·HCl	0.50	0.50		0.25		0.50		
Sucrose				10,000.0	10,000.0	10,000.0		
Thiamine·HCl	0.40	0.40		2.50		5.00		
Grams of powder to prepare 1 liter	4.510	1.180	3.050	12.100	13.200	14.210	3.200	2.880
pH±0.5 at RT	3.50	5.00	4.25	4.50	4.25	4.25	4.50	4.25

PLANT TISSUE CULTURE BASAL SALTS & MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Tobacco Callus Initiation Medium	Tobacco Root Initiation Medium	Tobacco Shoot Multiplication Medium	Tobacco Shoot & Root Medium	TM4G Basal Medium	Westvaco WV3 Medium	Westvaco WV5 Medium	White Basal Salt Mixture
COMPONENT	**T856**	**T861**	**T864**	**T867**	**T868**	**W863**	**W865**	**W898**
Ammonium Nitrate	1,650.0	1,650.0	1,650.0	1,650.0	320.0		700.0	
Ammonium Phosphate, Monobasic					230.00			
Ammonium Sulfate					130.0			
Boric Acid	6.20	6.20	6.20	6.20	6.20	31.00	31.00	1.50
Calcium Chloride, Anhydrous	333.000	333.000	333.000	333.000	113.250	452.880	452.880	
Calcium Nitrate								208.500
Cobalt Chloride·$6H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	0.0250	
Cupric Sulfate·$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0250	0.2500	0.2500	0.0010
Na_2 EDTA·$2H_2O$					18.65			
Ferric Sodium EDTA	36.700	36.700	36.700	36.700		36.700	36.700	
Ferrous Sulfate·$7H_2O$					13.90			2.50
Magnesium Sulfate, Anhydrous	181.000	181.000	181.000	181.000	122.124	903.790	903.790	351.620
Manganese Sulfate·H_2O	16.900	16.900	16.900	16.900	16.900	15.160	15.160	5.310
Sodium Molybdate(VI)·$2H_2O$	0.250	0.250	0.250	0.250	0.250	0.250	0.250	
Molybdenum Trioxide								0.001
Potassium Chloride						656.790	718.670	65.000
Potassium Iodide	0.830	0.830	0.830	0.830	0.830	0.830	0.830	0.750
Potassium Nitrate	1,900.00	1,900.00	1,900.00	1,900.00	1,900.00	910.06	1,084.06	80.00
Potassium Phosphate, Monobasic, Anhydrous	170.0	170.0	170.0	170.0		270.0	270.0	
Sodium Nitrate								16.500
Sodium Phosphate Monobasic·H_2O								200.0
Zinc Sulfate·$7H_2O$	8.600	8.600	8.600	8.600	9.200	8.600	8.600	3.000
D-Biotin					0.050			
Casein, Enzymatic Hydrolysate	1,000.0	1,000.0	1,000.0	1,000.0				
Folic Acid					0.500			
Glycine	2.000	2.000	2.000	2.000	2.500			
Indole-3-acetic Acid	2.000	3.000		0.030				
Kinetin	0.200		1.000	1.000				
myo-Inositol	100.0	100.0	100.0	100.0	100.0	1,000.0	1,000.0	
Nicotinic Acid	0.50	0.50	0.50	0.50	5.00			
Pyridoxine·HCl	0.50	0.50	0.50	0.50	0.50			
Thiamine·HCl	0.40	0.40	0.40	0.40	0.50	0.40	0.40	
Grams of powder to prepare 1 liter	5.410	5.410	5.410	5.410	2.990	4.290	5.220	0.930
pH±0.5 at RT	5.50	5.50	5.50	5.50	4.25	4.75	4.75	4.75

ORCHID MEDIA FORMULATIONS

All components expressed in mg/L	BM-1 Terrestrial Orchid Medium w/out Agar	BM-1 Terrestrial Orchid Medium w/ Agar	BM-2 Terrestrial Orchid Medium w/ Agar	BM-2 Terrestrial Orchid Medium w/out Agar	Fast Terrestrial Orchid Medium	Ichihashi New Phalaenopsis (NP) Medium	Knudson C Orchid Medium	Knudson C Modified Plus Orchid Medium
COMPONENT	**B138**	**B141**	**B142**	**B470**	**F522**	**I365**	**K400**	**K425**
Aluminum Chloride•$6H_2O$					0.030			Proprietary Formulation
Ammonium Nitrate					167.0	82.0	500.0	
Ammonium Sulfate						303.900	500.000	
Boric Acid	10.000	10.000	10.000	10.000	1.000	3.100		
Calcium Nitrate					40.100	637.600	347.200	
Cobalt Chloride•$6H_2O$	0.0250	0.0250	0.0250	0.0250		0.0125		
Cupric Sulfate•$5H_2O$	0.0250	0.0250	0.0250	0.0250	0.0300	0.0125		
Na2 EDTA•$2H_2O$	37.250	37.250	37.250	37.250		37.300		
Ferric Chloride					1.000			
Ferric Sodium EDTA					17.000			
Ferrous Sulfate•$7H_2O$	27.850	27.850	27.850	27.850		27.800	25.000	
Magnesium Nitrate•$6H_2O$						256.400		
Magnesium Sulfate, Anhydrous	100.000	100.000	100.000	100.000	19.800		122.125	
Manganese Sulfate•H_2O	25.000	25.000	25.000	25.000	0.800	11.200	5.682	
Sodium Molybdate(VI)·$2H_2O$	0.2500	0.2500	0.2500	0.2500		0.1250		
Nickel Chloride•$6H_2O$					0.030			
Potassium Chloride					167.000		250.000	
Potassium Iodide					0.0100	0.4150		
Potassium Nitrate						424.0		
Potassium Phosphate, Dibasic					83.0			
Potassium Phosphate, Monobasic, Anhydrous	300.00	300.00	300.00	300.00		462.70	250.00	
Zinc Sulfate•$7H_2O$	10.000	10.000	10.000	10.000	1.000	4.300		
Agar		5,000.0	6,000.0		7,000.0			
6-Benzylaminopurine			0.200	0.200				
D-Biotin	0.050	0.050	0.050	0.050	0.010			
Casein, Enzymatic Hydrolysate	500.000	500.000	500.000	500.000				
Fructose					5,000.0			
Folic Acid	0.500	0.500	0.500	0.500				
Gellan Gum, CultureGel™						3,000.000		
L-Glutamine	100.000	100.000	100.000	100.000				
Glycine	2.000	2.000	2.000	2.000		2.000		
myo-Inositol	100.00	100.00	100.00	100.00		100.00		
Nicotinic Acid	5.000	5.000	5.000	5.000	0.100	0.500		
Peptone from Meat					1,670.0			
Pyridoxine•HCl	0.500	0.500	0.500	0.500		0.500		
Sucrose	20,000.0	20,000.0	20,000.0	20,000.0	11,670.0	20,000.0	20,000.0	
Thiamine•HCl	0.500	0.500	0.500	0.500		0.100		
Yeast Extract					2,000.0			
Grams of powder to prepare 1 liter	21.22	26.22	27.22	21.22	27.84	25.35	22	79.11
pH±0.5 at RT	5.5	5.5	5.5	5.25	NS	4.75	4.5	NS

NS = No Specification Established

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ORCHID MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Lindemann Orchid Medium	Malmgren Modified Terrestrial Orchid Medium w/out Sucrose, Agar, & Pineapple Powder	Murashige Cattleya Orchid Multi. Medium	Malmgren Modified Terrestrial Orchid Medium w/out Sucrose & Agar	Malmgren Modified Terrestrial Orchid Medium	Mitra Replate/ Maintenance Medium	Orchid Maintenance Medium w/out Charcoal
COMPONENT	**L472**	**M482**	**M507**	**M534**	**M551**	**M579**	**O139**
Aluminum Chloride•6H_2O	0.056						
Ammonium Nitrate			1650.0				825.0
Ammonium Sulfate	1,000.0					100.000	
Boric Acid	1.014		6.200			0.600	3.100
Calcium Chloride, Anhydrous			333.000				166.000
Calcium Nitrate	347.200					100.000	
Calcium Phosphate, Tribasic		75.000		75.000	75.000		
Cobalt Chloride•6H_2O			0.0250				0.0125
Cobalt Nitrate•6H2O						0.0500	
Cupric Sulfate•5H_2O	0.0190		0.0250			0.0500	0.0125
Na2 EDTA•2H_2O		37.260		37.260	37.260	22.300	37.300
Ferric Citrate	4.400						
Ferric Sodium EDTA			36.700				
Ferrous Sulfate•7H_2O		27.800		27.800	27.800	16.700	27.850
Magnesium Sulfate, Anhydrous	58.620	97.676	181.000	97.676	97.676	250.000	90.350
Manganese Chloride•4H2O						0.400	
Manganese Sulfate•H_2O	0.052	1.540	16.900	1.540	1.540		8.450
Sodium Molybdate(VI)·2H_2O			0.2500			0.0500	0.1250
Nickel Chloride•6H_2O	0.031						
Potassium Chloride	1,050.0						
Potassium Iodide	0.0990		0.8300			0.0300	0.4150
Potassium Nitrate			1900.0			180.0	950.0
Potassium Phosphate, Monobasic, Anhydrous	135.00	75.00	170.00	75.00	75.00		85.00
Sodium Phosphate, Monobasic						150.00	
Zinc Sulfate•7H_2O	0.565		8.600			0.050	5.300
Activated Charcoal		1,000.0		1,000.0	1,000.0	2,000.0	
Agar					7,000.0	7,000.0	
D-Biotin		0.050		0.050	0.050	0.050	
Casein, Enzymatic Hydrolysate		400.0		400.0	400.0		
Citric Acid, Anhydrous			150.000				
Folic Acid		0.500		0.500	0.500	0.300	
Glycine	2.000	2.000	2.000	2.000	2.000		
Indole-3-acetic Acid			0.300				
Indole-3-butyric Acid			1.750				
MES (Free Acid)							1000.0
myo-Inositol	100.00	100.00	100.00	100.00	100.00		100.00
α-Naphthaleneacetic Acid			1.750				
Nicotinic Acid	1.000	5.000	0.500	5.000	5.000	1.250	1.000
Pineapple Powder				20,000.0	20,000.0		
Peptone from Meat							2,000.0
Pyridoxine•HCI	1.000	5.000	0.500	5.000	5.000	0.300	1.000
Riboflavin						0.050	
Sucrose	20,000.0		20,000.0			20,000.0	20,000.0
Thiamine•HCI	10.000	10.000	10.000	10.000	10.000	0.300	10.000
Grams of powder to prepare 1 liter	22.71	1.84	24.57	21.84	28.84	29.82	25.31
pH±0.5 at RT	4.5	5.75	3.25	4.25	4.25	NS	5.25

NS = No Specification Established

ORCHID MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Orchid Maintenance/ Replate Medium	H1 Oat Medium	H2 Oat Medium	Orchid Multiplication Medium	PhytoTech Phalaenopsis Replate Medium	Orchid Maintenance Medium	Orchid Maintenance Medium	Orchid Seed Sowing Medium
COMPONENT	**O156**	**O612**	**O622**	**O753**	**P656**	**P658**	**P668**	**P723**
Ammonium Nitrate	825.0			825.0	Proprietary Formulation	825.0	825.0	412.5
Boric Acid	3.100			3.100		3.100	3.100	1.650
Calcium Chloride, Anhydrous	166.000			166.000		166.000	166.000	83.000
Calcium Nitrate		96.600	96.600					
Cobalt Chloride•$6H_2O$	0.0125			0.0125		0.0125	0.0125	0.0063
Cupric Sulfate•$5H_2O$	0.0125			0.0125		0.0125	0.0125	0.0063
Na2 EDTA•$2H_2O$	37.300			37.300		37.300	37.300	18.650
Ferrous Sulfate•$7H_2O$	27.850			27.850		27.850	27.850	13.930
Magnesium Sulfate, Anhydrous	90.350	23.900	23.900	90.350		90.350	90.350	75.180
Manganese Sulfate•H_2O	8.450			8.450		8.450	8.450	4.230
Sodium Molybdate(VI)·$2H_2O$	0.1250			0.1250		0.1250	0.1250	0.0625
Potassium Chloride		100.000	100.000					
Potassium Iodide	0.4150			0.4150		0.4150	0.4150	0.2075
Potassium Nitrate	950.0			950.0		950.0	950.0	475.0
Potassium Phosphate, Monobasic, Anhydrous	85.00	200.00	200.00	85.00		85.00	85.00	42.50
Zinc Sulfate•$7H_2O$	5.300			5.300		5.300	5.300	2.650
Activated Charcoal	2,000.0					2,000.0	2,000.0	1,000.0
Agar		7,000.0	7,000.0	7,000.0		8,000.0		8,000.0
Banana Powder	30,000.0							
6-Benzylaminopurine				2.000				
Glucose			2,000.0					
MES (Free Acid)	1000.0			1000.0		1000.0	1000.0	500.0
myo-Inositol	100.00			100.00		100.00	100.00	100.00
α-Naphthaleneacetic Acid				0.500				
Nicotinic Acid	1.000			0.500		1.000	1.000	1.000
Oats, Rolled		3,000.0	3,000.0					
Peptone from Meat	2,000.0			2,000.0		2,000.0	2,000.0	2,000.0
Pyridoxine•HCI	1.000			0.500		1.000	1.000	1.000
Sucrose	20,000.0	2,000.0		20,000.0		20,000.0	20,000.0	20,000.0
Thiamine•HCI	10.000			1.000		10.000	10.000	10.000
Yeast Extract		100.000	100.000					
Grams of powder to prepare 1 liter	57.31	12.52	12.52	32.30	61.31	35.31	27.31	32.74
pH±0.5 at RT	5.00	NS	NS	5.25	NS	5.25	5.25	5.75

NS = No Specification Established

ORCHID MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Orchid Seed Sowing Medium	Orchid Maintenance/ Replate Medium	PhytoTech Orchid Replate Medium	PhytoTech Orchid Replate Medium	PhytoTech Orchid Replate Medium	Orchid Multiplication Medium	Terrestrial (Cypripedium) Orchid Medium	Terrestrial (Cypripedium) Orchid Medium	Terrestrial (Cypripedium) Orchid Medium
COMPONENT	**P727**	**P748**	**P781**	**P782**	**P785**	**P793**	**T839**	**T842**	**T849**
Ammonium Citrate			Proprietary Formulation	Proprietary Formulation	Proprietary Formulation		19.000	19.000	19.000
Ammonium Nitrate	412.5	825.0				825.0			1400.0
Boric Acid	1.650	3.100				3.100	0.500	0.500	0.500
Calcium Chloride, Anhydrous	83.000	166.000				166.000			
Calcium Nitrate							400.000	600.000	400.000
Cobalt Chloride•6H_2O	0.0063	0.0125				0.0125			
Cupric Sulfate•5H_2O	0.0063	0.0125				0.0125	0.0250	0.0250	0.0250
Na2 EDTA•2H_2O	18.650	37.300				37.300			
Ferric Ammonium Citrate							25.000	25.000	25.000
Ferrous Sulfate•7H_2O	13.930	27.850				27.850			
Magnesium Sulfate, Anhydrous	75.180	90.350				90.350	97.699	97.699	97.699
Manganese Sulfate•H_2O	4.230	8.450				8.450	1.540	1.540	1.540
Sodium Molybdate(VI)·2H_2O	0.0625	0.1250				0.1250	0.0200	0.0200	0.0200
Potassium Chloride							100.000	100.000	100.000
Potassium Iodide	0.2075	0.4150				0.4150	0.1000	0.1000	0.1000
Potassium Nitrate	475.0	950.0				950.0	200.0	200.0	200.0
Potassium Phosphate, Monobasic, Anhydrous	42.50	85.00				85.00	200.00	200.00	200.00
Zinc Sulfate•7H_2O	2.650	5.300				5.300	0.500	0.500	0.500
Activated Charcoal		2,000.0							
Agar	8,000.0	7,000.0					6,000.0	6,000.0	6,000.0
Banana Powder		30,000.0							
6-Benzylaminopurine						2.000			
Casein, Enzymatic Hydrolysate							400.000	200.000	
D-Glucose, Anhydrous							20,000.0	20,000.0	20,000.0
MES (Free Acid)	500.0	1000.0				1000.0			
myo-Inositol	100.00	100.00				100.00			
α-Naphthaleneacetic Acid						0.500			
Nicotinic Acid	1.000	1.000				0.500			
Peptone from Meat	2,000.0	2,000.0				2,000.0			
Pyridoxine•HCI	1.000	1.000				0.500			
Sucrose	20,000.0	20,000.0				20,000.0			
Thiamine•HCI	10.000	10.000				1.000			
Grams of powder to prepare 1 liter	31.74	64.31	35.81	43.81	65.79	25.30	27.44	27.44	28.44
pH±0.5 at RT	5.50	5.00	4.75	5.00	4.75	5.25	5.50	5.50	5.25

ORCHID MEDIA FORMULATIONS (Cont.)

All components expressed in mg/L	Vacin & Went Modified Orchid Basal Salts	Vacin and Went Modified Orchid Medium	Vacin & Went Modified Orchid Medium	Vacin & Went Modified Orchid Medium
COMPONENT	**V505**	**V882**	**V891**	**V895**
Ammonium Sulfate	500.000	500.000	500.000	500.000
Calcium Phosphate, Tribasic	200.000	200.000	200.000	200.000
Na2 EDTA•2H_2O	37.260	37.260	37.260	37.260
Ferrous Sulfate•7H_2O	27.800	27.800	27.800	27.800
Magnesium Sulfate, Anhydrous	122.100	122.100	122.100	122.100
Manganese Sulfate•H_2O	5.600	5.600	5.600	5.600
Potassium Nitrate	525.0	525.0	525.0	525.0
Potassium Phosphate, Monobasic, Anhydrous	250.00	250.00	250.00	250.00
Agar				7,000.0
Sucrose			20,000.0	20,000.0
Thiamine•HCI		0.400	0.400	0.400
Grams of powder to prepare 1 liter	1.67	1.67	21.67	28.67
pH±0.5 at RT	5.75	5.75	5.75	5.75

VITAMIN FORMULATIONS

All components expressed in mg/L	Chu N6 Vitamin Solution (1000x)	Erikkson Vitamin Solution (1000x)	Gamborg Vitamin Solution (1000x)	Gamborg Vitamin Powder (1000x)	Kao & Michayluk Vitamin Solution (100x)	MS Vitamin Powder (1000x)
COMPONENT	**C149**	**E330**	**G219**	**G249**	**K421**	**M533**
p-Aminobenzoic Acid					2.0	
L-Ascorbic Acid					200.0	
D-Biotin					1.0	
Calcium Pantothenate					100.0	
Choline Chloride					100.0	
Cyanocobalamin, Vit B_{12}					2.0	
Folic Acid					40.0	
Glycine	2,000.0	2,000.0				2,000.0
myo-Inositol			100,000	100,000	10,000.0	100,000
Niacinamide					100.0	
Nicotinic Acid	500.0	500.0	1,000.0	1,000.0		500.0
Pyridoxine•HCI	500.0	500.0	1,000.0	1,000.0	100.0	500.0
Retinol, Vit. A					1.0	
Riboflavin					20.0	
Thiamine•HCI	1,000.0	500.0	10,000.0	10,000.0	100.0	100.0
Grams of powder to prepare 1 liter	N/A	N/A	N/A	11.2	N/A	103.1
pH±0.5 at RT	4.25	3.75	4.25	NS	NS	4.00

NS = No Specification Established

VITAMIN FORMULATIONS (Cont.)

All components expressed in mg/L	Morel & Martin Vitamin Solution (100x)	Morel & Wetmore Vitamin Solution (100x)	Nitsch & Nitsch Vitamin Solution (1000x)	Nitsch & Nitsch Vitamin Powder (1000x)	Staba Modified Vitamin Solution (100x)	Schenk & Hildebrandt Vitamin Powder (100x)
COMPONENT	**M587**	**M592**	**N603**	**N608**	**S743**	**S826**
p-Aminobenzoic Acid					50.0	
L-Ascorbic Acid						
D-Biotin	1.0	1.0	50.0	50.0	100.0	
Calcium Pantothenate	100.0	100.0			100.0	
Choline Chloride					100.0	
Cyanocobalamin, Vit B_{12}					0.150	
Folic Acid			500.0	500.0	50.0	
Glycine			2,000.0	2,000.0		
myo-Inositol	10,000.0	10,000.0	100,000.0	100,000.0	10,000.0	100,000.0
Niacinamide					200.0	
Nicotinic Acid	100.0	100.0	5,000.0	5,000.0		500.0
Pyridoxine•HCI	100.0	100.0	500.0	500.0	200.0	50.0
Retinol, Vit. A						
Riboflavin					50.0	
Thiamine•HCI		100.0	500.0	500.0	100.0	500.0
Grams of powder to prepare 1 liter	NA	NA	N/A	108.6	N/A	101.05
pH±0.5 at RT	NS	NS	NS	3.75	NS	4.00

NS = No Specification Established

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MICROBIOLOGY/PHYTOPATHOLOGY MEDIA FORMULATIONS							
All components expressed in mg/L	Bacteria Screening Medium 523	Corn Meal Agar	Czapek-Dox Broth	Czapek-Dox Agar	Hanahan's Broth	LB Agar (Luria-Bertani Agar)	LB Broth (Luria-Bertani Broth)
COMPONENT	**B129**	**C442**	**C443**	**C506**	**H289**	**L465**	**L475**
Ferrous Sulfate·$7H_2O$			10.00	10.00			
Magnesium Sulfate			500.00	500.00	2,400.00		
Magnesium Sulfate·7H2O	150.00						
Potassium Chloride			500.00	500.00	186.00		
Potassium Phosphate, Dibasic			1,000.00	1,000.00			
Potassium Phosphate, Monobasic	2,000.00						
Sodium Chloride					500.00	10,000.00	10,000.00
Sodium Nitrate			3,000.00	3,000.00			
Agar	8,000.00	7,000.00		15,000.00		12,000.00	
Casein, Enzymatic Hydrolysate	8,000.00						
Corn Meal, Yellow		15,000.00					
Sucrose	10,000.00		30,000.00	30,000.00			
Tryptone					20,000.00	10,000.00	10,000.00
Yeast Extract	4,000.00				5,000.00	5,000.00	5,000.00
Grams of powder to prepare 1 liter	32.15				49.69	37.00	25.00
pH±0.5 at RT	6.00				6.8-7.2	NS	NS

NS = No Specification Established

MICROBIOLOGY/PHYTOPATHOLOGY MEDIA FORMULATIONS (Cont.)							
All components expressed in mg/L	Leifert & Waites Sterility Test Medium	LB Broth, Lennox L Modification	LB Agar, Lennox L Modification	LB Broth Solution, Lennox L Modification	LB Broth Solution w/ Glycerol, Lennox L Modification	Malt Extract Broth	Malt Extract Agar
COMPONENT	**L476**	**L543**	**L552**	**L585**	**L591**	**M484**	**M498**
Ammonium Nitrate	825.00						
Boric Acid	3.10						
Calcium Chloride, Anhydrous	166.10						
Cobalt Chloride·6H_2O	0.01						
Cupric Sulfate·5H_2O	0.01						
Na2 EDTA	18.63						
Ferrous Sulfate·7H_2O	13.90						
Magnesium Sulfate	90.35						
Manganese Sulfate·H_2O	8.45						
Molybdic Acid (Sodium Salt)·2H_2O	0.13						
Potassium Iodide	0.42						
Potassium Nitrate	950.00						
Potassium Phosphate, Monobasic	85.00						
Sodium Chloride	2,000.00	5,000.00	5,000.00	5,000.00	5,000.00		
Zinc Sulfate·7H_2O	4.30						
Agar			12,000.00				15,000.00
D-Glucose, Anhydrous	5,000.00						
Glycerol (Density = 1.261 g/mL)					5.04		
Glycine (Free Base)	1.00						
Malt Extract						17,000.00	30,000.00
Meat Extract, LabLemco	7,000.00						
myo-Inositol	50.00						
Nicotinic Acid (Free Acid)	0.25						
Peptone from Meat	4,000.00					3,000.00	3,000.00
Pyridoxine·HCI	0.25						
Sucrose	15,000.00						
Thiamine·HCI	0.05						
Tryptone		10,000.00	10,000.00	10,000.00	10,000.00		
Yeast Extract	10,000.00	5,000.00	5,000.00	5,000.00	5,000.00		
Grams of powder to prepare 1 liter	45.20	20.00	32.00	NA	NA	20.00	48.00
pH±0.5 at RT	NS	NS	NS	NS	NS	NS	NS

NS = No Specification Established

MICROBIOLOGY/PHYTOPATHOLOGY MEDIA FORMULATIONS (Cont.)								
All components expressed in mg/L	Melin-Norkrans Medium, Modified	Nutrient Agar	Nutrient Broth	Oatmeal Agar	Peptone Water	Peptone Water, Buffered\	Potato Dextrose Broth	Potato Dextrose Agar
COMPONENT	**M580**	**N601**	**N611**	**O606**	**P632**	**P732**	**P762**	**P772**
Ammonium Phosphate, Monobasic	184.70							
Calcium Chloride, Anhydrous	50.00							
FeNaEDTA	20.00							
Magnesium Sulfate	35.80							
Potassium Phosphate, Monobasic	500.00					1,500.0		
Sodium Chloride	25.00				5,000.0	5,000.0		
Sodium Phosphate, Dibasic						3,500.0		
Agar	7,000.0	15,000.0		7,000.0				15,000.0
D-Glucose, Anhydrous							20,000.0	20,000.0
Malt Extract	3,000.0							
Meat Extract, LabLemco		3,000.0	3,000.0					
Oats, Rolled				3,500.0				
Peptone from Meat		5,000.0	5,000.0		15,000.0	10,000.0		
Potato Powder							4,000.0	4,000.0
Sucrose	10,000.0							
Thiamine·HCI	0.10							
Grams of powder to prepare 1 liter	20.82	23.00	8.00	10.50	20.00	20.00	24.00	39.00
pH±0.5 at RT	NS	NS	NS	NS	NS	NS	NS	NS

NS = No Specification Established

Microbiology/Plant Pathology Media Formulations (Cont.)						
All components expressed in mg/L	SOC Medium Solution	Terrific Broth Solution with Glycerol	Terrific Broth	Wilkins-Chalgren Agar	YEP Medium	YMB Medium
COMPONENT	**S657**	**T754**	**T760**	**W887**	**Y889**	**Y893**
Magnesium Sulfate	2,400.00					200.00
Potassium Chloride	186.00					
Potassium Phosphate, Dibasic		9,400.00	9,400.00			500.00
Potassium Phosphate, Monobasic		2,200.00	2,200.00			
Sodium Chloride	500.00			5,000.00	5,000.00	100.00
Agar				10,000.00	15,000.00	15,000.00
L-Arginine (Free Base)				1,000.00		
D-Glucose	3,600.00			1,000.00		
Glycerol (Density = 1.261 g/mL)		5.04				
Haemin				5.00		
Mannitol						10,000.00
Menadione				0.50		
Peptone from Meat					10,000.00	
Peptone from Gelatin				10,000.00		
Pyruvic Acid				1,000.00		
Tryptone	20,000.00	11,800.00	11,800.00	10,000.00		
Yeast Extract	5,000.00	23,600.00	23,600.00	5,000.00	10,000.00	400.00
Grams of powder to prepare 1 liter	NA	NA	47.00	43.01	40.00	26.20
pH±0.5 at RT	7.00	NS	NS	NS	NS	NS

NS = No Specification Established

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