

## **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 through 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  $\mu\text{mho/cm}$ .

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- $\mu\text{m}$  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  $\pm 0.5^\circ\text{C}$ ; 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 the 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  $\pm 3\%$ . 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)	B933	295.00	Measuring out biochemicals and media
1	pH meter (range 0-14 +/- 0.01; automatic temperature compensation 0-60° C; one or two point calibration)	P976	79.90	Measurement and adjustment of media pH
1	Hot Plate/Stirrer (7" x 7" ceramic top; variable heating range from ambient to 400° C; variable stirring speed from 50-150 rpm; chemically resistant)	H926	295.00	Mixing & heating media and stock
1	Refrigerator/freezer; capable of maintaining a refrigerator temperature of 2-6° C with a freezer temperature of approximately -20° C	NA		Storage of stock solutions, media, hormones
1	Laminar Flow Transfer Hood; incoming air should be HEPA filtered to remove 99.99% of particles larger than 0.3µm; 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 100 ft roll)	NA		Used to wrap instruments prior to sterilization, cover vessels
12	Beakers, 250 mL	B910	7.75	Mixing solutions
12	Beakers, 1000 mL	B931	13.75	Mixing solutions
6	Beakers, 2000 mL	B939	32.25	Mixing solutions and media
6	Beakers, 4000 mL	B960	46.25	Mixing solutions and media
4 ea	Bottle, Water; pre-labeled for use in dispensing water; 500 mL capacity	B987	6.00	Rinsing instruments, beakers, transplants from tissue culture
4 ea	Bottle, Isopropyl Alcohol; pre-labeled for use in dispensing isopropyl alcohol; 500 mL capacity	B974	6.00	Rinsing sterile hood work surfaces
1 case	Bottle, 100 mL; Type 1 borosilicate glass with volume graduations; supplied with 33-340 black polypropylene cap with rubber liner; non-sterile	NA		Storage of stock solutions, sterile distilled water, media
1 case	Bottle, 500 mL; Type 1 borosilicate glass with volume graduations; supplied with 33-340 black polypropylene cap with rubber liner; non-sterile	NA		Storage of stock solutions, sterile distilled water, media
2	Brushes, flask or bottle	NA		Cleaning glassware
1 case	Culture tubes, 25 x 150 mm, borosilicate glass; 500 tubes/ case	C930	146.50	Starting cultures in Stage I
1 case	Culture tube racks; holds 40, 25 mm culture tubes; withstands temperatures up to 121° C	C908	90.50	Holding culture tubes
500	Closures, for 25 mm culture tubes, 500 each	C945	139.25	Sealing culture tubes
1 case	Culture vessel, baby food jar; glass culture vessel; autoclavable; uses Magenta B Cap (C903) as closure; 6 oz; 100/ case	C006	44.50	Culture vessel for maintaining plant cultures

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US\$)	ITEM FUNCTION
1 case	Culture vessels, baby food jar; glass culture vessel; autoclavable; uses Magenta B Cap (C903) as closure; 4 oz height; 100/ case	C900	38.50	Culture vessel for maintaining plant cultures
1 case	Magenta B Caps; autoclavable closure for baby food jars; fits both C904 and C900; clear polypropylene closure; 100/ case	C903	215.50	Closure for baby food culture vessel
2 cases	Culture vessels; autoclavable culture vessel and lid made from clear polypropylene; round vessel measures; 250/ case	C913	173.00	Culture vessel for maintaining plant cultures
1 gal	Detergent	NA	.	Cleaning glassware
1 case	Culture dishes, disposable, sterile, 100 x 15 mm	D940	75.50	Sterile surface for cutting explants (for Stage I cultures)
1 gal	Chlorine bleach (sodium hypochlorite)	NA	.	Surface sterilize explants
1	Erlenmeyer flask, wide mouth, 1000 mL	F985	18.50	Mixing media
1	Erlenmeyer flask, wide mouth, 2000 mL	F986	25.50	Mixing media
1	Erlenmeyer flask, 4000 mL	NA	.	Mixing media
1	Erlenmeyer flask, 6000 mL	NA	.	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
3	Forceps, dressing; 10" length, serrated, stainless steel	F952	35.50	Transferring tissue
3	Forceps, bayonet; stainless steel	F957	76.50	Transferring tissue
3	Forceps; stainless steel, very fine point, 5.5" length	F084	.	Transferring tissue
1	Graduated cylinder, glass or plastic, 10 mL	C962	3.50	Preparing stock solutions
1	Graduated cylinder, glass or plastic, 100 mL	C964	6.25	Preparing stock solutions
1	Graduated cylinder, glass or plastic, 1000 mL	C968	20.25	Preparing stock solutions
1 pkg	Lab markers, assorted colors (10)	NA	.	Labeling cultures
1 roll	Parafilm (4" x 250 ft)	S911	36.50	Wrapping culture closures
100 ea	Pipets, 1 mL, graduated, sterile, disposable, individually wrapped	P990	15.50	Measuring out stock solutions
100 ea	Pipets, 5 mL, graduated, sterile, disposable, individually wrapped	P993	28.50	Measuring out stock solutions
100 ea	Pipets, 10 mL, graduated, sterile, disposable, individually wrapped	P994	26.50	Measuring out stock solutions
100 ea	Pipets, 25 mL, graduated, sterile, disposable, individually wrapped	P995	61.50	Measuring out stock solutions
1	Pipet pump, electric	P091, P092	260.00	For safely measuring liquids
1 pkg	Gloves, hot or autoclave; provides protection up to 350° F	G089, G092	.	Safely removing hot items from autoclave
2 ea	Scalpel handle; No 3: 5" length; stainless steel	S963	31.00	Cutting explants
2 ea	Scalpel handle; No 3L: 8" length; stainless steel	S973	37.00	Cutting explants
1 box	Scalpel blades; No 10; stainless steel; individually wrapped; sterile; 100/box	S970	59.50	Cutting explants
1 ea	Scoop, large plastic 5-3/4" x 9" bowl	NA	.	Measuring large volumes of biochemicals
1 pkg	Scoop, medium, 30 cm length, 2/pkg	NA	.	Measuring small to medium amounts of biochemicals

QTY	ITEM DESCRIPTION	PHYTOTECH PRODUCT NO.	APPROX. COST (US\$)	ITEM FUNCTION
2 ea	Spatula, stainless steel blade, wooden handle, 4" wide x 19/32" thick	NA		Measuring small to medium amounts of biochemicals
1 pkg	Spatula, micro; 8" length, flat nickel stainless steel	NA		Measuring small to medium amounts of biochemicals; rounded ends, 2" end length
1 ea	Sterilizer, pressure cooker; operates between 116-126° 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° 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 120 V (S636) or 240 V (S637)	S636	415.50	Sterilizes instruments in hood between transfers
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	NA		Retrieving stir bars from mixing vessel
1 roll	Tape, autoclave indicator; impregnated to show the word "Autoclaved" after 15 minutes of exposure at 121° 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	NA		Labeling cultures, storage bottles, media vessels, etc.
2 ea	Thermometer; -20-150° C temperature range	NA		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
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	26.50	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	32.75	Measuring chemicals
500 ea	Weighboats, large, plastic; can be utilized for liquid or solid samples; 5-1/2" x 5-1/2" x 1"	W881	75.75	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.