## Setting Up a Tissue Culture Lab:

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, some lead-lined to resist acids and alkalis, draining boards, and racks, and have access to demineralized water, distilled water, and double-distilled water. Space for drying ovens or racks, automated dishwashers, acid baths, pipette washers and driers, and storage cabinets should also be available in the washing area.

**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, distilled and double-distilled water, Bunsen burners with a gas source, refrigerators and freezers for storing stock solutions and chemicals, a microwave or a 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, and exact step-by-step routine should be developed for media preparation and a complete checklist required of all media preparers even for the simplest media.

The water used in preparing media must be of the utmost purity and highest quality. Tap water is unsuitable because it may contain cations (ammonium, calcium, iron, magnesium, sodium, etc.), anions (bicarbonates, chlorides, fluorides, phosphates, etc.), microorganisms (algae, fungi, bacterial), gases (oxygen, carbon dioxide, nitrogen), 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 9% 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 bacteria-free filtered air prevents nonfiltered air and particulate matter 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 95% ethyl alcohol when in use. This type of unit is used when relatively few transfers are required.

**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 cultural 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 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 ±3 percent. 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 Required to Start a Plant Cell Culture Laboratory

| QTY | ITEM DESCRIPTION | PHYTOTECH CATALOGUE NO. | APPROX. COST | 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) | B 933 | 295.00 | 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) | P 976 | 74.95 | 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) | H 926 | 295.00 | Mixing & heating media and stock |
| 1 | Refrigerator/freezer; capable of maintaining a refrigerator temperature of 0-5$^0$ C with a freezer temperature of approximately –20$^0$ C | NA | 750.00 | Storage of stock solutions, media, hormones |
| 1 | Laminar Flow Transfer Hood; incoming air should be HEPA filtered to remove 99.99% of particles larger that 0.3µm; should meet or exceed the Class 100 Clean Standard 209D; maintain a flow of 90 fpm +/- 20% at static pressures of 0.6-1.2" | NA | 3500.00 | Provide a sterile atmosphere to transfer cultures |
| | 4 liter Isopropyl alcohol | NA | 10.00 | Used to sterilize instruments and work areas |
| 1 roll | Aluminum foil, heavy duty; (18" x100 ft roll) | NA | 2.50 | Used to wrap instruments prior to sterilization, cover vessels |
| 12 | Beakers, 250 mL | B 910 | 7.75 | Mixing solutions |
| 12 | Beakers, 1000 mL | B 931 | 13.75 | Mixing solutions |
| 6 | Beakers, 2000 mL | B 939 | 32.25 | Mixing solutions and media |
| 6 | Beakers, 4000 mL | B 960 | 46.25 | Mixing solutions and media |
| 4 ea | Bottle, Water; pre-labeled for use in dispensing water; 500 mL capacity | B 974 | 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 | B 987 | 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 | | 24.00 | 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 | | 50.00 | Storage of stock solutions, sterile distilled water, media |
| 2 | Brush, flask or bottle | | | Cleaning glassware |

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|---|---|---|---|---|
| 1 case | Culture tubes, 25 x 150 mm, borosilicate glass; 500 tubes/case | C 930 | 146.50 | Starting cultures in Stage I |
| 1 case | Culture tube racks; holds 40, 25 mm culture tubes; withstands temperatures up to $121^0$ C | C 908 | 90.50 | Holding culture tubes |
| 500 | Closure, for 25 mm culture tubes, 500 each | C 925 | 57.50 | Sealing culture tubes |
| 1 case | Culture vessel, baby food jar; glass culture vessel; autoclaveable; uses Magenta B Cap (C 903) as closure; 110 mm height; 100/case | C 904 | 42.50 | Culture vessel for Maintaining plant cultures |
| 1 case | Culture vessel, baby food jar; glass culture vessel; autoclavable; uses Magenta B Cap (C 903) as closure; 72 mm height; 100/case | C 900 | 32.50 | Culture vessel for Maintaining plant cultures |
| 1 case | Magenta B Cap; autoclavable closure for baby food jars; fits both C 904 and C 900; clear polypropylene closure; 100/case | C 903 | 215.50 | Closure for baby food culture Vessel |
| 2 cases | Culture vessel; autoclavable culture vessel and lid made from clear polypropylene; round vessel measures; 250/case | C 913 | | Culture vessel for maintaining plant cultures |
| 1 gal | Detergent | NA | 5.75 | Cleaning glassware |
| 1 case | Culture dishes, disposable, sterile, 100 x 25 mm | D 940 | 173.50 | For Stage I cultures sterile surface for cutting explants |
| 1 gal | Disinfectant, commercial brand | NA | 2.00 | Disinfects explants |
| 1 | Erlenmeyer flask, wide mouth, 1000 mL | F 985 | 18.50 | Mixing media |
| 1 | Erlenmeyer flask, wide mouth, 2000 mL | F 986 | 25.50 | Mixing media |
| 1 | Erlenmeyer flask, 4000 mL | | | Mixing media |
| 1 | Erlenmeyer flask, 6000 mL | | | Mixing media |
| 1 case | 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; 12 units/case | NA | 63.00 | Sterilization of heat liable stock solutions |
| 3 | Forceps, dressing; 10” length, serrated, stainless steel | F 952 | 30.75 | Transferring tissue |
| 3 | Forceps, bayonet; stainless steel | F 957 | 71.85 | Transferring tissue |
| 5 | Forceps; stainless steel, very fine point, 6” length | | | Transferring tissue |
| 1 | Graduated cylinders, glass or polypropylene, 10 mL | C 962 | 3.50 | Preparing stock solutions |
| 1 | Graduated cylinders, glass or polypropylene, 100 mL | C 964 | 6.25 | Preparing stock solutions |
| 1 | Graduated cylinders, glass or polypropylene, 1000 mL | C 968 | 20.25 | Preparing stock solutions |
| 1 pkg | Lab markers, assorted colors (10) | NA | | Labeling cultures |

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|---|---|---|---|---|
| 1 roll | Parafilm (4" x 250 ft) | S 911 | 36.50 | Wrapping culture Closure |
| 100 | Pipets, 1 mL, graduated, sterile, disposable, individually wrapped | P990 | 14.50 | Measuring out stock solutions |
| 100 | Pipets, 5 mL, graduated, sterile, disposable, individually wrapped | P993 | 26.25 | Measuring out stock solutions |
| 100 ea | Pipets, 10 mL, graduated, sterile, disposable, individually wrapped | P994 | 24.95 | Measuring out stock solutions |
| 100 ea | Pipets, 25 mL, graduated, sterile, disposable, individually wrapped | P995 | 58.25 | Measuring out stock solutions |
| 1 | Pipet pump, electric | NA | 190.00 | For safely measuring liquids |
| 1 pkg | Gloves, hot or autoclave; provides protection up to $350^0$ F | NA | 6.00 | Safely removing hot items from autoclave |
| 2 ea | Scalpel handle; No 3: 5" length; stainless steel | S963 | 69.75 | Cutting explants |
| 2 ea | Scalpel handle; No 3L: 8" length; stainless steel | S973 | 83.75 | Cutting explants |
| 1 box | Scalpel blades; No 10; stainless steel; individually wrapped; sterile; 100/box | S970 | 56.25 | Cutting explants |
| 1 | Scoop, large plastic 5-3/4" x 9" bowl | NA | 11.00 | Measuring large volumes of biochemical's |
| 1 pkg | Scoop, medium, 30 cm length, 2/pkg | NA | 34.65 | Measuring small to medium amounts of biochemical's |
| 2 ea | Spatula, stainless steel blade, wooden handle, 4" wide x 19/32" thick | NA | 11.00 | Measuring small to medium amounts of biochemical's |
| 1 pkg | Spatula, micro; 8" length, flat nickel stainless steel | NA | 25.25 | Measuring small to medium amounts rounded ends, 2" end length; of biochemical's |
| 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 | NA | 500.00 | Sterilizing media and instrument |
| 1 ea | Sterilizer, Autoclave; operates at $121^0$ C with dial for fast or slow exhaust; 0-60 minute timer; stain-less unit with 66 41 cm chamber | NA | 6500.00 | Sterilizing media and instruments |
| 1 ea | Sterilizer, dry heat with glass beads 120 V (S636) or 240 V (S637) | S636 | 395.00 | Sterilizes instruments in hood between transfers |
| 1 pkg | Stirring bars, magnetic; Teflon covered; leakproof, seamless and contain permanent Alnico V magnets | NA | 58.00 | Used for mixing stock solutions and in media preparation |
| 1 ea | Stir bar retriever; contains an Alnico V magnet sealed in polyethylene; 18" length | NA | 9.00 | Retrieving stir bars from mixing vessel |

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| 1 roll | Tape, autoclave indicator; impregnated to show the word "Autoclaved" after 15 minutes of exposure at $121^0$ C | T 998 | 3.95 | Identifying autoclaved media |
| 1 roll | Tape, Label; all purpose, self adhesive tape can be written on with pen or permanent marker | | | Labeling cultures, storage bottles, media vessels, etc. |
| 2 ea | Thermometer; -20-$150^0$ C temperature range | NA | 40.00 | Measuring temperature of liquids and culture room |
| 1 | Timer; electronic, countdown timer alarm, stopwatch feature | NA | 30.00 | Timing sterilization and general lab use |
| 1 case | Towels; commercial, single fold | | 13.00 | Can be sterilized to provide sterile work surface for cutting explants, gen lab use |
| 500 ea | Weighing boats, plastic; can be utilized for liquid or solid samples; 1-5/8" x 1-5/8" x 5/16" | W 879 | 26.50 | Measuring chemicals |
| 500 ea | Weighing boats, plastic; can be utilized for liquid or solid samples; 3-5/16" x 3-5/16" x 1" | W 880 | 32.75 | Measuring chemicals |
| 500 ea | Weighing boats, plastic; can be utilized for liquid or solid samples; 5-1/2" x 5-1/2" x 1" | W 881 | 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 seven to eight times 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 all 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, washed, and rinsed between washes before being used for tissue culture. Other glassware commonly required in a tissue culture facility includes beakers, volumetric flasks, pipettes, and graduated cylinders.