

PRODUCT CIRCULAR SPECIFICATION

Star*Pac® Plant Tissue Culture System Instruction Sheet

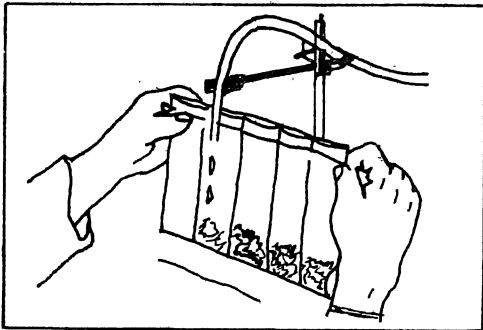
INTRODUCTION:

The Star*Pac® system is a versatile, disposable tool for plant tissue culture application. The stage of development and size of the culture governs the configuration best suited for a particular application. For demonstration purposes, the five chamber design will be featured in the procedure. (Note: The five chamber unit is equivalent to five 25mm x 150mm test tubes, five caps and five pieces of moisture barrier/sealing film)

FILLING METHODS:

Direct pipetting can be used to add media to chambers.

- 1) Open the upper portion of the chamber with a slight twist between the thumb and forefinger.
- 2) Insert the pipet into the opening and dispense the media. (5 -10 ml per chamber for the five chamber unit are recommended).



Automatic pipetting is the preferred filling method.

- 1) Set the filling volume: 5-10 ml per chamber for the five chamber unit.
- 2) Open the upper portion of the chamber with a slight twist between the thumb and forefinger.
- 3) Insert the filling nozzle into the first opening and dispense media (i.e., press foot pedal or finger pad control). Systematically move the succeeding chambers into position and fill accordingly.

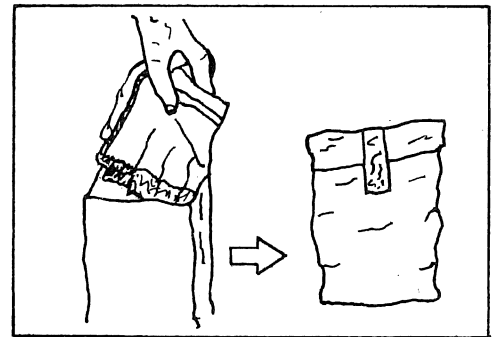
STERILIZATION:

The recommended method for sterilizing the Star*Pac® is autoclaving (steam under pressure).

- 1) Insert 10-15 filled Star*Pacs® into a paper bag or other autoclavable, steam permeable container.
- 2) Fold the top of the bag down several times and seal with a piece of autoclave sensitive tape.
- 3) Load the autoclave with the paper bags, and operate under customary settings for liquid sterilization (i.e., 121 C, 15 psi, 15 minutes)

Note: Temperatures exceeding 124 C are unnecessary for proper sterilization, and can cause container failure.

- 4) After autoclaving, the sealed bags should be taken to a clean counter or hood to allow the media to cool before use.

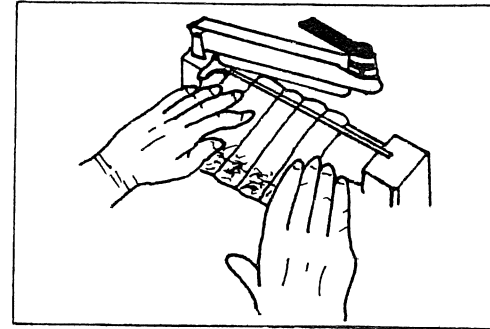


SEALING THE CHAMBERS:

The recommended sealing method involves the use of an 8 or 12 inch heat sealer. The sealer should be left under the hood, at one of the far ends of the work area.

Caution: The heat sealer is an electrical device and should be handled with the proper precautions. Operating and troubleshooting instructions are provided by the sealer manufacture.

- 1) Have the unit turned on and ready to use prior to critical aseptic operations. The recommended heat setting for the unit is between 4 and 5, although the optimal setting may vary from instrument to instrument.
- 2) Lift the handle of the sealer and place the open end of the chambers across the sealing strip. (Use the printing at the top of the five chamber unit as a reference point for the seal)
- 3) Lower the handle of the heat sealer over the top of the Star*Pac®. Use your fingers to keep the Star*Pac® in position while sealing.
Warning: Do not place fingers under the sealing handle.
- 4) To ensure a good seal, observe the red indicator light on the heat sealer. Do not lift the handle too quickly: allow a few seconds for the seal to cool before lifting the handle. An indication of a good seal is a uniform, clear line across the top of the Star*Pac®.



CULTURE CONDITIONS:

Once the Star*Pacs® are sealed, the cultures can be moved out of the hood, and into the culture area. Since the walls of the sealed Star*Pac® are gas permeable and liquid impermeable, there are virtually no requirements for a precisely controlled, humidified environment. This feature also greatly reduces the risk of contamination, and eliminates the need for a sterile culture area.

The light scattering qualities of the Star*Pac® enables one to place the cultures in an upright position (as opposed to conventional techniques involving slanted tube and flask cultures).

SUBCULTURING:

To minimize the risk of contamination, all culture transfers should be performed under a laminar flow hood. Additionally, aseptic techniques should be followed to avoid costly contamination risk.

- 1) Conveniently arrange the following materials under the hood:

sterile scalpels, sterile glass plates or petri dishes, sterile forceps, electric sterilization unit of Bunsen burner, wash bottle with filtered alcohol, sterile whipes, media-filled chambers, surface-sterilized chambers containing established cultures, and heat sealer.

2) Aseptically, remove the media filled Star*Pac® from the paper bags and set them aside in the hood.

3) Surface sterilize the chambers containing the established cultures by:

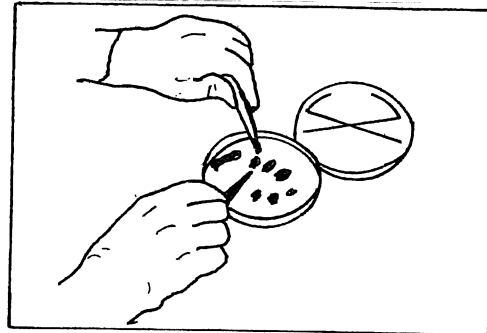
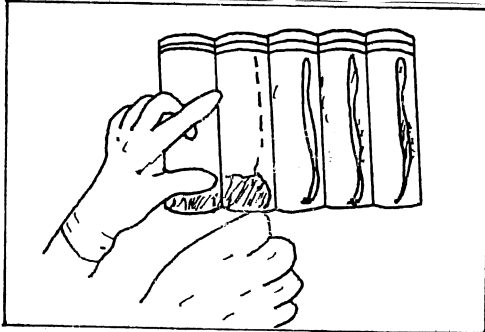
a) total submersion in 10% hypochlorite solution for five minutes or

b) spraying down with filtered 70% isopropyl alcohol

If established cultures do not exist, and a direct explant is being used, it must be surface sterilized and trimmed according the established laboratory protocols. (In this case, eliminate steps 3,4,6 and 7)

4) Place the wet, disinfected chambers under the hood on a sterile paper towel to drain.

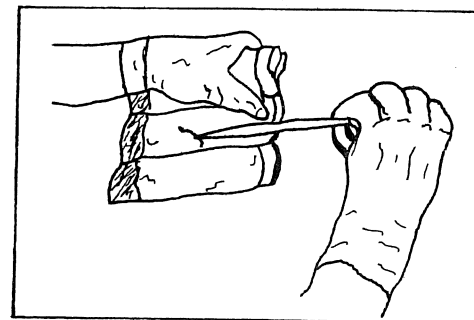
5) Open the sterile work surface (glass plate or petri dish) and set it aside.



6) Open the chamber containing the established culture by cutting through the side of the chamber with a sterile scalpel. Care should be taken not to cut through both layers of the chamber.

7) Aseptically remove the culture and place it on the sterile work surface. Divide and size the tissues appropriately.

8) With one hand, open a chamber containing media, using the same twisting motion used in filling the bags. With the other hand pick up a piece of tissue with long, sterile forceps and insert it into the chamber and position it on the media. Note: The sterile forceps can be inserted into the chamber and moved from side to side to facilitate opening of the chamber prior to inserting the culture.



The flexible Star*Pac® can be placed in trays, clipped on lines, or left free standing under the proper light conditions used for routine culture (i.e., typically cool white light approx. 12 inches above Star*Pacs®: 16 hours on/8 hours off.) Additionally, the collapsible design of the Star*Pac® provides space savings in limited culture areas.

Sealed Star*Pacs® containing advanced stages of plant development can also be cultured under reduced light conditions in the greenhouse, prior to planting in soil.

