Is contamination in your Plant Tissue Cultures still worrying you???

Biogenuix®

Plant Cell TECHNOLOGY

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Try PPM™ Today!!

Why Plant Preservative Mixture (PPM™)

Plant Preservation Mixture (PPM™) is a patented product, specially formulated for prevention and protection of plant tissue cultures from endogenous and exogenous contamination – without damaging the ex-plant itself.

- Non-toxic to most plant species at optimal concentrations
- Ideal for both Endogenous & Exogenous Fungal / Bacterial contamination
- Maintains clean cultures at very low concentrations
- Thermostable- can be added directly to media before autoclaving
- Environmentally safe & does not cause resistance unlike antibiotics

Introduction:

- PPM is a heat stable preservative/biocide which can be used to effectively prevent or reduce microbial contamination in plant tissue culture.
- At optimum doses, PPM, which stands for Plant Preservative Mixture, is an extremely effective Preservative/Biocide, yet does not impair in vitro seed germination, callus proliferation and callus regeneration.
- Despite the most stringent use of sterile techniques, the contamination of plant cell and plant tissue cultures remain a persistent problem that can result in losses ranging from small number of cultures to the loss of whole batches.
- PPM prevents the germination of both bacteria and fungi spores. It is heat stable and therefore can be autoclaved with the media.
- PPM can be, and should be used as standard ingredients in Plant Tissue Culture Media, and is also substantially less expensive than commonly used antibiotics.
- While PPM was principally designed to inhibit airborne contamination, waterborne contamination and contamination introduced from human contact, It can also in many cases be used to reduce endogenous contamination

Mechanism Of Action

- PPM is a broad-spectrum preservative and biocide, which kills bacteria and fungi cells, prevents germination of spores, and in higher concentrations, can eliminate explants of endogenous contamination.
- The active ingredients of PPM penetrate the fungus or the bacterium cell wall and inhibit the activity of key enzymes within the central metabolic cycles such as the citric acid cycle and the electron transport chain.
- PPM may also inhibit the transport of monosaccharides and amino acids from the medium into the fungus or bacterium cells
- As in any biocide, a critical ratio of PPM molecules per microbial cell is needed to eliminate bacteria and fungi. Keep in mind that a given volume of PPM does has a constant number of PPM molecules while the number of spores introduced to tissue culture via endogenous contamination is highly varied. Therefore, explants should not be "sqeezed" into a beaker. There should be enough volume for free movement of the solution around the explant material.

Advantages of PPM Over Antibiotics

- PPM is broad-based and effective against fungi.
- PPM is less expensive than antibiotics, making it affordable for wide and routine use.
- Since PPM targets and inhibits multiple enzymes, the formation of resistant mutants towards PPM is very unlikely.
- PPM is heat stable and in general can be autoclaved with media.

PPM™ significantly simplifies the tissue culture working procedures as follows:

- *Media containing PPM™ may be dispensed outside the laminar flow hood (LFH) exposed to the ambient air. The plates should be covered soon after agar solidification. In the event that media dispensing is done by a pump, we recommend passing autoclaved hot water through the hoses prior to and after media dispensing.*
- *Heat sensitive or heat stable liquid media containing PPM™ does not need to be filter sterilized or autoclaved provided that it will be stored in sterile containers and that the stock solutions are not contaminated. In rich media containing 200 mg/liter or more of amino acids or proteins, it is recommended to filter the media with the PPM™.*
- *If working in the LFH the utensils (forceps or scalpels) do not need to be flamed. They should be periodically dipped in 70% alcohol. The LFH does not need to be certified and the work can be done as well outside the LFH on a clean surface for a period not exceeding 1 hour.*
- *PPM™ is less effective when exposed to high density of bacteria or fungi spores found regularly on seed's coat. For in vitro germination, seeds should be conventionally surface sterilized with EPA registered bleach. Therefore, in the presence of PPM™ (in the germination medium), the seeds can be rinsed under tap water in a non-sterile strainer and left to dry preferably in the LFH. If the utensil ends have touched active bacteria, fungi culture or otherwise suspected of being contaminated, they should be sterilized by autoclave or by use of an electric heating element.*
- *General Dosage levels: With the exception of endogenous contamination, the recommended dose range is 0.05%-0.2%. (For callus proliferation, organogenesis and embryogenesis, the recommended range is 0.05-0.075%.) To eliminate higher endogenous contamination densities, higher doses of PPM are needed (see paragraph 6 below).*
- *Endogenous Contamination: (a) For explants: gently and routinely shake / stir 1 cm. long explants (or shorter) for 4-12 hours in 4-5% v/v PPM™ solution supplemented as above with full MS strength basal salts without pH ing and without Tween 20. Without rinsing, insert into a medium supplemented with 0.05 - 0.1% PPM™ for herbaceous plants and 0.2% PPM™ for woody plants. Note: Paragraphs **6(b)** through 10 below are intended for ornamental plants only. **(b)** For tubers, bulbs and scales: shake / stir the entire tuber / bulb / scale in bleach. Rinse with water (can be done under non-sterile conditions). Slice the tuber / bulb / scale to thin slices. Shake / stir for 12-24 hours in 4 - 5% PPM™ solution supplemented with full strength basal salts without pH ing and Tween 20. Without rinsing, insert into a medium supplemented with 0.1 - 0.2% PPM™.*
- *In cases where the above protocols do no yield satisfying results (especially thick explants, highly infested explants, seeds), we recommend the following: **(a)** Shake / stir the explants in water (1hr for soft tissues and 2 hr for hard tissues). **(b)** Shake / stir the explants in (50%) PPM™ supplemented with full strength MS basal salts (without pH ing and without Tween 20) for 5 -10 minutes. **(c)** Without rinsing, insert the explants into the medium. In fungal contamination, the addition of PPM™ to the medium is optional. However, with bacterial or mixed contamination, the addition of 0.05 - 0.2% PPM™ to the medium in the first month is essential. Do not discard highly oxidized explants as approximately 50% of the explants will recover within 4 - 6 week*
- *To eliminate Agrobacterium: After co-cultivation, rinse the leaf discs with water. Dip (entirely) the transfected discs in a 100% PPM™ solution (supplemented with full strength basal salts) for approximately 2 minutes. Blot the discs between two sterile paper towels and place onto a medium supplemented with full-strength of the commonly used antibiotics. After 3 weeks, transfer to the medium with solely PPM at 0.05 0.075%.*

General Notes:

1. For the first transfer following the sterilization with PPM™, we recommend to insert the explants entirely into a semi-solid medium.

2. The 50% PPM™ solution can be reused but is not recommeded. The number of uses depends on the volume of the explants treated and the inoculum density. Keeping the 50% PPM™ solution stored at 4ºC prolongs its activity. If necessary, prepare two PPM™ solutions: one to disinfect endogenous contamination and the second, to disinfect "in-culture" contamination. The second solution should be filtered after each treatment, using 0.2 micrometer Millipore. The filtration process can be done in non-sterile atmosphere. A single filter can be used for the entire "lifespan" of the solution.

3. In cases where the treatment with 50% PPM™ is still insufficient, full strength PPM™ (100%) can be used. The treatment with 100% PPM™ is similar to the one described above for 50% PPM™, however, the exposure time should not exceed 10 minutes.

PPM Usage

PPM has been known to be very effective in a large variety of explant TCs. We have end-user suggested protocols readily available for the following explants:

Regular Plants

- Cauliflower
- Traubia modesta
- Curcuma, Kaempferia and Zingiber
- Petunia hybrida
- Cotton
- Ilex paraguariensis
- Hydrangea macrophylla (Thunb.) Ser. and Hydrangea paniculata
- Black kangaroo paw
- T. modesta
- Carnivorous Plants (Dionaea)
- Ficus carica
- Cortaderia
- Peperomia pelucida
- Gerbera, Orchids, Perennials
- Cephalotus follicularis
- Native hawaiian seeds
- Lonicera
- Norway Spruce
- Hornwort
- Centaurea spp.
- Vaccinium
- Dionaea muscipula
- Aloe
- Magnolia
- Avocado
- Berries
- Potato
- Asperagus
- Tomato
- Rice
- Fern
- Sweet potato
- Maize
- Soybean
- Canola
- Juglans
- Petunia
- Alfalfa
- Medicago
- Switchgrain
- Rice
- Ciban
- Tomato
- Clusia
- Kalanchoe
- Poplar
- Orchids
- Nepenthes
- Rosa hybrida
- Bambusa
- Orchid
- Hosta
- Nepenthes
- Sarracenia
- Citrus
- Cortaderia
- OIL
- Canola
- Elm
- Wasabi
- Amelanchier
- Blueberry
- Ornamentals
- Cucumber
- Banana
- Wheat
- Cantharanthus
- Maize
- Anubias
- Nepenthes
- Hosta
- Raspberry
- Blueberry
- Impatiens
- Orchids

For more information on detailed usage protocols kindly contact us on http://biogenuix.com/contact/

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