PPM™ (Plant Preservative Mixture) is a heat stable preservative/biocide that, based on the dose, effectively prevents or reduces microbial contamination in plant tissue culture.

At optimum doses, PPM™, does not impair in vitro seed germination, callus proliferation or callus regeneration.

Despite the most stringent use of sterile techniques and aseotic conditions, the contamination of plant cell and plant tissue cultures remain a persistent problem.

PPM™ prevents the germination of both bacteria and fungi spores. Because its heat stable, it can be autoclaved with media.

PPM™ can be used as a standard ingredient in plant tissue culture media. PPM is less expensive than antibiotics and can prevent fungal contamination simultaneously.

PPM is both biostatic and biocidal. In addition to inhibiting airborne, waterborne and human contact contamination, it also can be used to reduce endogenous contamination.

The principal PCT scientist involved in the development of the PPM™ application is Dr. Assaf Guri. Dr. Assaf Guri holds degrees in genetics, applied genetics and plant breeding from the Hebrew University in Jerusalem and Michigan State University in the US. Before joining Plant Cell Technology, Inc., Assaf worked with the Volcani Agricultural Research Center in Israel, Michigan State University in East Lansing, Michigan and DNAP in New Jersey.

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 endogenous contamination in explants.

Previous research has shown that 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. Our data indicates that 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.

ADVANTAGES 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 unlikely.
- PPM™ is heat stable and in general can be autoclaved with media.

IDENTIFICATION

Chemical Name: PPM™ (Plant Preservative Mixture)
Chemical Family: Proprietary
CAS#: Proprietary (Several #s)

Synonyms: N/A
DOT Information: Not Corrosive (pH = 3.7)

Hazard	Rating	Scale
Oral toxicity	4	4=INSIGNIFICANT/NONE
Eye/Skin Toxicity/Irritation	3 (Caution)	3=MODERATE
Fire	4	2=HIGH
Reactivity	4	1=EXTREME/CORROSSIVE

The above rating is Plant Cell technology's toxicity rating. It incorporates some EPA criteria.

COMPANY IDENTIFICATION

Plant Cell Technology, Inc.
1823 Jefferson Street, NW
Washington, DC 20036, USA
+1.202.788.8522 ext. 123

1. PHYSICAL DATA

Appearance: Clear
Color: Amber to clear
State: Liquid
Odor Characteristic: Mild, inoffensive odor
pH: 3.0 – 4.0
Solubility in Water: Completely soluble

2. INGREDIENTS

PPM™ is a proprietary chemical product.

3. FIRE AND EXPLOSION HAZARD DATA

Flash Point

- Auto-ignition Temperature Not Applicable
- Lower Explosive Limit Not Applicable
- Upper Explosive Limit Not Applicable

Unusual Hazards

- Combination generates toxic fumes of the following: hydrogen chloride, nitrogen oxides, and sulfur oxides.

Extinguishing Agents

- Use extinguishing media appropriate for surrounding fire.

Personal Protective Equipment

- Wear self-contained breathing apparatus (pressure-demand MSHA/NIOSH approved or equivalent) and full protective gear.

Special Procedures

- Use water spray to cool containers exposed to fire. Minimize exposure. DO NOT breathe fumes. Contain run-off.

4. HEALTH HAZARD DATA

Inhalation

- Inhalation of vapor or mist can cause the following: irritation to nose and throat.

Eye Contact

- Material can cause the following: corrosion to dyes – permanent, irreversible eye injury.

Skin Contact

- Skin irritation effects can be delayed for hours. Material can cause the following: irritation to the skin, allergic contact dermatitis.

Ingestion

- Material is harmful if swallowed.

5. FIRST AID MEASURES

Inhalation

- Move subject to fresh air. Give artificial respiration if breathing has stopped.

Ingestion

- If swallowed, IMMEDIATELY call and see a physician. Never give anything by mouth to an unconscious person.

Eye and Skin Contact

- IMMEDIATELY flush eyes with a large amount of water for at least 15 minutes. Wash affected skin areas thoroughly with soap and water. Remove and wash contaminated clothing thoroughly. Do not take clothing home to be laundered. Discard contaminated shoes, belts and other articles made of leather. Get prompt medical attention.

Note to Physician:

- MATERIAL HAS a pH of 3.0-4.0. It may be advised to induce vomiting. Possible mucosa damage may contraindicate the use of gastric lavage. Measures against circulatory hock and convulsions may be necessary.

6. REACTIVITY DATA

- This material is considered stable under specified conditions of storage, shipment and/or use.
- Thermal decomposition may yield the following: hydrogen chloride, sulfur dioxide, oxides of nitrogen.
- Hazardous Polymerization: Production will not undergo polymerization.
- Incompatibility: Avoid contact with the following: oxidizing agents, reducing agents, amines, mercaptans.

7. SPILL OR LEAK PROCEDURES

Dilute with 100x water. Rinse decontaminated solution to a chemical sewer.

8. SPECIAL PROTECTION INFORMATION

Personal Protection

- Wear compatible, chemically resistant gloves.
- Material has a pH of 3.0 – 4.0. If exposed to material during clean-up operations **IMMEDIATELY** remove all contaminated clothing and wash exposed skin areas with soap and water. See SECTION 6, First Aid Measures, for further information.
- Protective clothing made of the following material should be worn to avoid skin contact: butyl, rubber or nitrite.

Engineering Controls (Ventilation)

- Use local exhaust ventilation with a minimum capture velocity of 150 ft. /min. (0.75 m/sec.) at the point of dust or mist evaluation. Refer to the current edition of Industrial Ventilation: A Manual or Recommended Practice published by the American Conference of Governmental Industrial Hygienists for information on the design, installation, use and, maintenance of exhaust systems.

Other Protective Equipment

- Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower.

9. HANDLING AND STORAGE

Storage Conditions

- The maximum recommended storage temperature for this material is 55° C/131° F. The minimum recommended storage temperature for this material is 0° C/32° F. Store in a well-ventilated area. Do not store this material in containers made of steel.

Handling Procedures

- See Exposure Controls/Personal Protection prior to handling. Since emptied container retain product residue (vapors and/or liquid) follow all MSDS and label warning even after container is emptied.

10. REGULATION INFORMATION

- **Workplace Classification:** This product is considered non-toxic.

11. TOXICOLOGICAL INFORMATION Oral LD50 – Rat: 30g/Kg

- **Eye/Skin Irritation – rabbit:** moderate irritation/insult, reversible damage
- **Sensitization Data:** Human – Allergic contact dermatitis possible.

12. ECOLOGICAL INFORMATION

- Not Available.

13. DISPOSAL CONDITIONS

- Dispose of PPM™-containing media as you would non-PPM™ media. Items that have been exposed to full strength PPM™ should be rinsed liberally (100x) in water. See Item #8 above.

14. TRANSPORTATION INFORMATION

- US DOT Hazard Class = Non-Toxic. Classified as a skin irritant.

The information contained herein relates only to the specific material identified. Plant Cell Technology, Inc. believes that such information is accurate and reliable as of the date of this material safety data sheet, but no representation, guarantee or warranty express or implied, is made as the accuracy, reliability, or completeness of the information. Our company urges persons receiving this information to make their own determination as to the information's suitability and completeness for their particular application.

Additional Information

PROTOCOLS & PROCEDURES

The procedures described below are generic. Slight modifications might be needed for your specific plant species. For assistance, contact Dr. Assaf Guri at guri1@erols.com

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

1. 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.

2. 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™.

3. 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.

4. 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.

5. **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).
6. **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™.

7. 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 weeks.

Note

Refer to notes 2 and 3 below.

8. To decontaminate "in culture" contaminated plant material (rescue treatment):

(Note: The culture should not be left visibly contaminated longer than one week.)

(a) Clean the material mechanically using a soft tooth-brush under a stream of tap water. Shake / stir in a 50% PPM™ solution (diluted with sterile water) for 5 - 15 minutes. For bacterial or mixed contamination

we recommend to lower the solution pH to the range of 2.8 - 3.2 by mixing 1:1 full strength PPM™ (100%) with 0.6 gr./liter Citric acid solution (use sterile water).

(b) Without rinsing insert into a medium with 0.05 - 0.2% PPM™ for at least one month. Keep the culture away from high light intensities for the first 10 days. As mentioned above, don't discard oxidized explants. Wait 4 - 6 weeks as approximately 50% should recover.

In some cases the fungal or the bacterial spores are located deep within the ex-plants beyond PPM's reach. In such cases, and after the water-soaking period, slice the ex-plants along and then stir/shake in 50% PPM for 5 -15 minutes.

THROUGH ALL THE ABOVE STERILIZATION PROCESSE(S), ENSURE THAT THE PPM PROFUSELY REACHES THE ENTIRE SURFACE OF THE EX-PLANT.

9. **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 recommended. 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.

Summary

PPM™ most definitely will facilitate the work in any plant tissue laboratory and should significantly increase technician and laboratory productivity. However, conditions in each lab may vary which could have a bearing on the effectiveness of PPM™. It is advisable that staff follows the above guidelines initially and adjust parameters accordingly.

When used as recommended:

- PPM™ is effective against airborne contamination, waterborne contamination and contamination introduced from human contact.
- If used correctly, PPM™ will eliminate endogenous contamination from explants.
- At recommended doses (0.5 - 2ml/l); PPM™ does not impair in vitro seed germination, callus proliferation, callus regeneration, and axillary or adventitious buds' induction.

Safety and Handling - See MSDS

DO NOT CONCENTRATE THE MATERIAL

DISPOSAL INFORMATION:

Dispose of media containing PPM™ in the same manner in which you dispose of media without PPM™. In an emergency, contact the following numbers: 1 (202) 271-0328 or +1.800.746.8535. A toxicological assessment has been performed by a qualified toxicologist. This assessment is available upon request. For more information on PPM™, or to request test results contact: **Tel:** 1.202.778.8522 ex. 0, **Fax:** 1.202.429.9812