

Directions of Use

Plant Preservation Mixture (PPM)

PPM is a broad-spectrum preservative and biocide. PPM is an excellent preservative agent that can be used in research and commercial laboratories to inhibit the growth of, or kill bacteria and fungi in plant tissue culture growth media. It targets fundamental enzymes in the Krebs cycle and in the Electron Transport Chain. Depending on the dose level, PPM is both biocidal (>2ml/L of media) and Biostatic (<2ml/L of media). When diluted with plant growth media (5-20 ml/L) PPM/liter growth media is effective as a micro-biocide against non-human health pathogenic organisms.

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 a pump dispenses the media, we recommend passing autoclaved hot water through the hoses before and after dispensing media.
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. Working in the LFH the utensils (forceps or scalpels) do not need to be flamed. They may be periodically dipped in 70% alcohol. The LFH does not need to be certified and the work can also be done outside the LFH on a clean surface for a period not exceeding 2 - 3 hours.
4. PPM comes in an acidic liquid solution (pH 3.8) and should be stored at 4°C. The recommended dose is 0.5 - 2.0 ml of PPM per liter of medium. Higher doses are required to treat endogenous contamination or to obtain Agro-bacteria free plant material.
5. PPM is less effective when exposed to high density of bacteria or fungi spores found regularly on a 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. Protoplast isolation solution should be sterilized mechanically through Millipore filters with the PPM. 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.

6. Endogenous Contamination: Plant tissue culture media containing PPM at doses of 5-20 ml/l can be used to eliminate endogenous contamination in seeds and plant-explants. In such cases, the seeds or the explants should be treated with an EPA registered plant disinfectant. After rinsing with DD water, explants or buds should be embedded or placed in autoclaved semisolid or liquid medium respectively. The proper media such as callus proliferation or regeneration can be used with only $\frac{1}{4}$ strength of the inorganic salts, supplemented with 5-20 ml/l PPM/media mixture. After 2-5 days the explants can be transferred without rinsing into a similar media (full strength inorganic salts) supplemented with at least 0.5 ml/l PPM at 20-24 degrees centigrade. Seeds can be transferred to germination medium (full strength of inorganic salts) supplemented with 0.5 ml/l PPM after 5-10 days.

It is up to the researchers to determine the optimal combination of PPM doses and time exposure. Different plant types and different explant sources are highly varied in their response to PPM.