

Plant Tissue Culture

Antibacterial, Antifungal & Selection Agents



Antibiotics

Avoidance or prevention of microbial contamination in plant tissue culture is critical. Microbial contamination is one of the major challenges in plant tissue culture. Microorganisms infecting plant tissue culture leads to depletion of nutrients in the medium and reduces chances of survival of the plant culture.

Antibiotics are used for the treatment and prevention of bacterial contamination. They specifically act either by killing or inhibiting growth of bacterial cells either by inhibition of cell wall synthesis or nucleic acid metabolism or protein synthesis. Antifungals are the chemical agents that kill or inhibit the growth of fungi and are used for the treatment and prevention of fungal contaminants.

Selection at cellular level has been practiced for desirable traits and success has been achieved in several crop plants. The potential of antibiotics as selection agents has been very well realized in plant transformation systems. A suitable selecting agent is the one which favours the growth of transformed cells and inhibits growth of untransformed plant cells. The concentration of selecting agent varies widely depending on the sensitivity of plant species. The sensitivity of plant cells to selection agent depends upon genotype, explant type, developmental stage and culture conditions.

Antibiotics such as Carbenicillin, Cefotaxime and Kanamycin Sulphate are the most commonly used selection agents in plant transformation protocols due to broad spectrum activity against bacteria and a low toxicity profile. Others include Hygromycin-B, Neomycin, G-418 disulfate salt (Geneticin disulphate), Paromomycin sulphate and Timentin.

It is always advisable to determine the least effective dose of antibiotics, as excess dose might induce phytotoxicity in the plant cells.

Quality Control Parameters

All our products undergo stringent quality control required to maintain consistency and quality. A statistically valid number of samples are withdrawn from each batch as per the defined procedure. We have documented procedures for quality control in accordance with GMP in process as well as finished products.

We have developed in-house testing criteria for all our products and have defined release criteria. Each lot is passed only if the product conforms to the release criteria. For customized products, additional quality control testing is available on request. Following are some parameters included in the routine testing protocols.

Quality Parameter	Description
Appearance	Individual product is tested for Color, Texture, Nature, Consistency
Solubility	Individual product's solubility is tested in the solvents that they are soluble in
FTIR	Infrared absorption and emission spectra are measured and are matched with the standard spectrum
Assay	Qualitative/ Quantitative measurement of the analyte is done by carrying out different kinds of assays like HPLC/NaOH/GC/NT/AT titration depending on the product
Melting Range	Purity of a substance and amount of impurities present in a sample is determined
Water (K.F.)	Water content is determined in the specified product
Specific Rotation	Optical purity of the product is determined by measuring the specific rotation of the compound
Potency Testing	Potency is a measure of antimicrobial activity expressed in terms of the amount required to produce an effect of given intensity. A highly potent antimicrobial evokes a given response at low concentrations. Potency depends on both the affinity and efficacy
Plant Tissue Culture Test	Antimicrobial properties of antimicrobial is assessed by adding the agent in the culture medium in optimum concentration that does not produce any phytotoxicity or necrotic effects on plant cultures

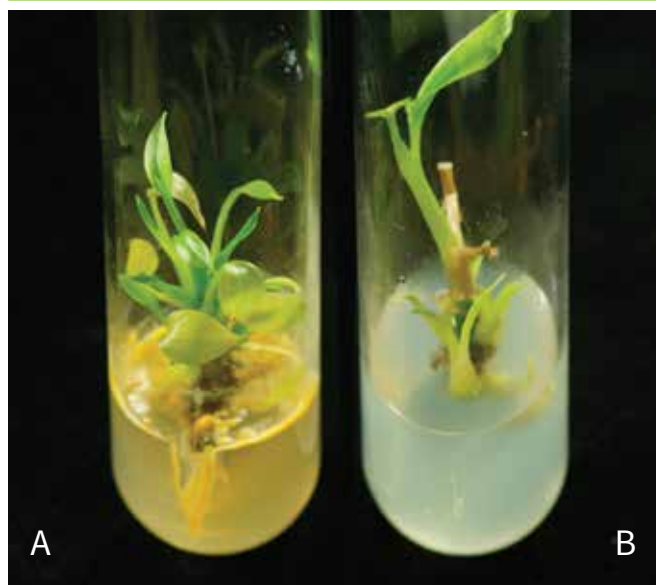
Antibacterial

PRODUCT INFORMATION

Product Name	Code	Packing	Recommended usage	Solubility	Mode of Action / Antibacterial Spectrum
Amoxycillin	PCT1107	1 gm 10 gm 25 gm	50 µg/ml	Ammonium hydroxide/ Water	Highly effective against the gram positive and gram negative bacteria and inhibits bacterial cell wall synthesis.
Amoxycillin : clavulanic acid potassium salt (5:1)	PCT1115	2 gm 10 gm	300 µg/ml	Water	Inhibits cell wall synthesis and crosslinking of peptidoglycan. Clavulanic acid is a specific inhibitor of β -lactamase and protects amoxicillin against the inactivation of β -lactamase.
Ampicillin sodium salt	PCT1101	1 gm 5 gm 10 gm 25 gm	100 µg/ml	Water	Inhibits cell wall synthesis by inactivating transpeptidases on inner surface of bacterial cell membrane. Acts against gram positive and gram negative bacteria and is a semi synthetic derivative of penicillin.
Ampicillin solution (100 mg/ml)	PCT1507	1x20 ml 1x100 ml			
Bacitracin (50,000 U/VL)	PCT1109	1 vL 10x1 vL 25x1 vL	150 µg/ml	Water	Interferes with the cell wall biosynthesis in gram positive bacteria.
Cephataxime sodium salt	PCT1103	1 gm 5 gm 10 gm 25 gm	100 µg/ml	Water	Inhibits bacterial cell wall synthesis and is highly effective against gram negative bacteria. Highly resistant against β -lactamase activity and is nontoxic to plant cells.
Cephataxime solution (100 mg/ml)	PCT1502	1x20 ml 5x20 ml			
Cephataxime solution (250 mg/ml)	PCT1505	1x20 ml 5x20 ml 1x100 ml			
Chloramphenicol	PCT1117	5 gm 25 gm 100 gm	50 µg/ml	EtOH	Inhibits mitochondrial and chloroplast protein synthesis. Effective against gram-negative and gram -positive bacteria.
Erythromycin	PCT1123	1 gm 5 gm 25 gm	150 µg/ml	Alcohol	Inhibits protein synthesis and has bacteriostatic action against gram positive bacteria.
Gentamicin sulphate	PCT1118	1 gm 5 gm 10 gm 25 gm	100 µg/ml	Water	Misreads codons by binding to 30S ribosomal subunit, blocking the translocation of peptidyl-tRNA from acceptor site to donor site. Effective against many gram negative bacteria and some strains of staphylococci.

PRODUCT INFORMATION

Product Name	Code	Packing	Recommended usage	Solubility	Mode of Action / Antibacterial Spectrum
Polymyxin B sulphate (100,000 U/VL)	PCT1125	1x1 mu 5x1 mu 25x1 mu	100 U/ml	Water	Acts by binding to the membrane phospholipids and disrupts bacterial cytoplasmic membrane. Active against gram-negative bacteria, especially <i>Pseudomonas</i> species.
Rifampicin	PCT1119	1 gm 5 gm 25 gm 100 gm	50 µg/ml	MeOH/DMSO	Inhibits initiation of RNA synthesis by binding to β-subunit of RNA polymerase. Active against gram positive bacteria but less active against gram negative bacteria.
Streptomycin sulphate	PCT1120	5 gm 25 gm 100 gm	100 µg/ml	Water	Inhibits prokaryote protein synthesis by miscoding or binding to the S12 protein of the 30S ribosomal subunit. Bactericidal in action against many gram negative bacteria.
Tetracycline hydrochloride	PCT1126	5 gm 25 gm 100 gm	50 µg/ml	Water	Inhibits binding of 30S ribosomes and protein synthesis by preventing access of aminoacyl tRNA to acceptor site on the mRNA-ribosome complex. Alters cell membrane and leads to intracellular components leakage from bacterial cells. Bacteriostatic activity against gram positive and gram negative bacteria.
Vancomycin hydrochloride	PCT1114	500 mg 1 gm 5 gm 25 gm	80 µg/ml	Water	Inhibits formation of peptidoglycan polymers of the bacterial cell wall.

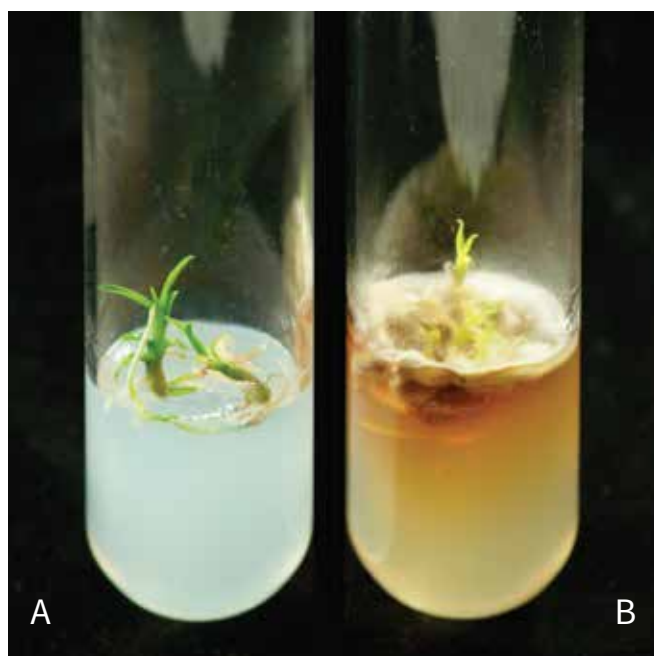


- A. Bacterial contaminated *Anthurium* sp. in MS medium without Cephotaxime (PCT1103).
- B. Healthy *Anthurium* sp. in MS medium containing 50µg/ml of Cephotaxime (PCT1103).

Antifungal

PRODUCT INFORMATION

Product Name	Code	Packing	Recommended usage	Solubility	Mode of Action
Amphotericin B	PCT1108	1 gm 5 gm 10 gm	2.5 µg/ml	DMSO	Effective against many fungi, yeast and molds. Interferes with permeability of cell membrane of sensitive fungi and yeasts.
Carbendazim	PCT1121	1 gm 5 gm 25 gm	160 µg/ml	DMF/HCl	Inhibits the formation of mitotic microtubules in fungi.
Cycloheximide	PCT1122	1 gm 5 gm	20 µg/ml	EtOH	Inhibits translation in eukaryotes resulting in cell growth arrest and cell death. Used as an antimycotic against yeasts, molds and fungi.
Miconazole nitrate	PCT1110	1 gm 5 gm 25 gm	20 µg/ml	MeOH	Interferes with ergosterol synthesis.
Nystatin (10,000,00 U/VL)	PCT1112	1 vL 10x1 vL 25x1 vL	100 U/ml	DMF/DMSO	Interferes with permeability of the cell membrane of fungi and yeasts.



- A. Healthy *Orchid* sp. in MS medium containing 2.5µg/ml of amphotericin (PCT1108).
- B. Fungal contaminated *Orchid* sp. in MS medium without amphotericin (PCT1108).

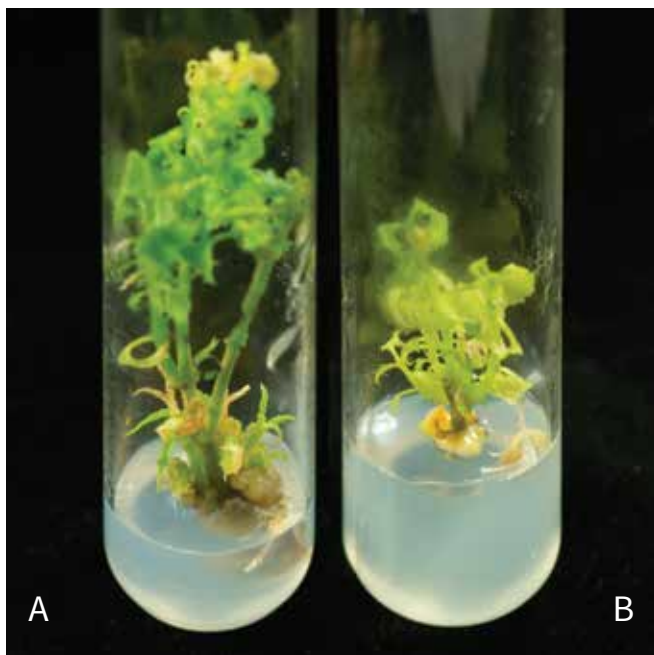
Antibacterial and Antifungal

Besides conventional antimicrobials, PCT1106, an Antimicrobial supplement is a broad spectrum chemically synthetic biocide that acts as an antibacterial as well as antifungal agent. Advantages of antimicrobial supplement over conventional antibiotics include :

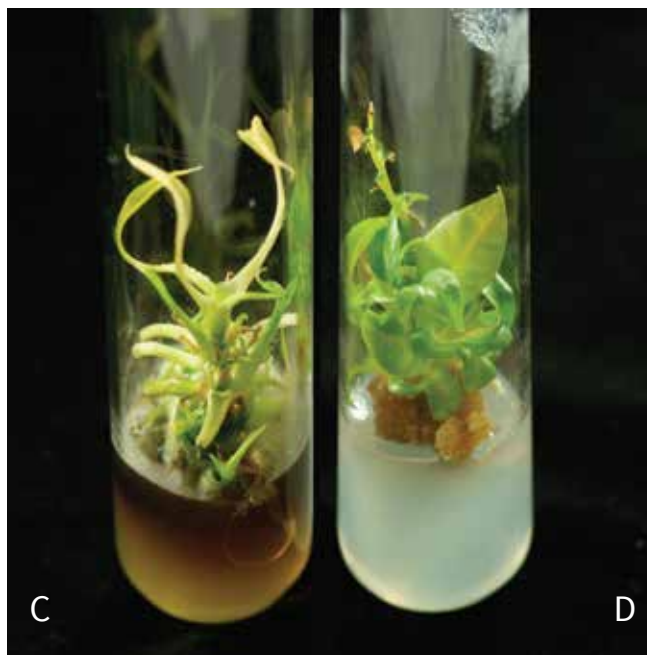
- Antimicrobial supplement is economical for use.
- It efficiently targets the bacterial as well as fungal cells and maintains clean cultures at very low concentrations.
- The antimicrobial supplement can be autoclaved easily and is user friendly.

PRODUCT INFORMATION

Product Name	Code	Packing	Recommended usage	Solubility	Mode of Action
Antimicrobial supplement	PCT1106	50 ml 100 ml	0.075%	Water	Has a wide range of activity and is effective against various gram negative bacteria, gram positive bacteria, fungi and yeasts.



- A. Healthy *Ficus* sp. in Woody Plant medium containing 0.075% of Antimicrobial supplement (PCT1106).
- B. Bacterial contaminated *Ficus* sp. in Woody Plant medium without Antimicrobial supplement (PCT1106).



- C. Fungal contaminated *Spathiphyllum* sp. in Gamborg medium without Antimicrobial supplement (PCT1106).
- D. Healthy *Spathiphyllum* sp. in Gamborg medium containing 0.05% of Antimicrobial supplement (PCT1106).

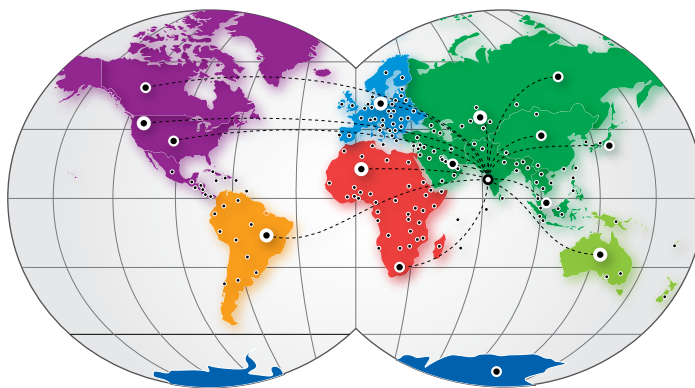
Selection Agents

PRODUCT INFORMATION					
Product Name	Code	Packing	Recommended usage	Solubility	Mode of Action / Antibacterial Spectrum
Carbenicillin disodium salt	PCT1102	250 mg 1 gm 5 gm 10 gm 25 gm	500 µg/ml	Water	Used for the elimination of agrobacterium tumefaciens during plant transformation. Nontoxic to plant cells and is a derivative of penicillin.
Carbenicillin solution (100 mg/ml)	PCT1501	1x20 ml 5x20 ml			
G-418 disulfate salt (Geneticin disulphate)	PCT1116	1 gm 5 gm	50 µg/ml	Water	Inhibits protein synthesis and is a derivative of neomycin.
Hygromycin – B	PCT1104	100 mg 250 mg 1 gm	200 µg/ml	Water	Used for selection and maintenance of prokaryotic and eukaryotic cells that contain hygromycin resistance gene. The resistance gene is a kinase that inactivates hygromycin B through phosphorylation.
Hygromycin – B solution (50 mg/ml)	PCT1503	1x20 ml 5x20 ml			
Kanamycin acid sulphate	PCT1105	1 gm 5 gm 25 gm 50 gm	50 µg/ml	Water	Acts by binding to the 70S ribosomal subunit, inhibiting translocation and eliciting miscoding. Effective against gram positive and gram negative bacteria. Used as selection agent in transformation studies.
Kanamycin solution (50 mg/ml)	PCT1504	1x20 ml 1x100 ml			
Neomycin sulphate	PCT1111	5 gm 25 gm 100 gm	500 µg/ml	Water	Acts by binding to the 30S and 50S subunits, causing miscoding and inhibition of initiation and elongation during protein synthesis. Effective against gram positive and gram negative bacteria.
Neomycin sulphate solution (10 mg/ml)	PCT1506	1x20 ml 5x20 ml			
Paromomycin sulphate	PCT1124	1 gm 5 gm	50 µg/ml	Water	Inhibits protein synthesis in protozoa and has a similar spectrum to that of neomycin. Also used to select genetically transformed plants and plant cells.
Timentin	PCT1113	2 gm	200 µg/ml	Water	Eliminates agrobacterium from the post transformation studies and is a broad spectrum semi synthetic penicillin.
Timentin solution (100 mg/ml)	PCT1508	1x20 ml 1x100 ml			

Note :

The recommended usage given in the table may vary for different plant species depending on their toxic sensitivity.

Stock solutions should be stored at recommended temperature.



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