Technical Information

Tobacco Callus Initiation Medium

With Vitamins, Casein hydrolysate, IAA, Kinetin And Without Sucrose, Agar

Product Code: PT1088

Application: Tobacco Callus Initiation Medium has been developed on the basis of Murashige and Skoog medium for the *in vitro* culture of *Nicotiana tabacum* species, family *Solanaceae*. The formulation is a nutrient blend of inorganic salts, vitamins, amino acid and plant growth regulators.

Tobacco Callus Initiation medium provides all essential macroelements and microelements. Casein hydrolysate is a source amino acid which leads to granular and compact callus. Potassium nitrate and ammonium nitrate act as nitrogen sources and promote morphogenesis. Potassium dihydrogen phosphate serves as a source of phosphate and helps in cell proliferation. Microelements like Boron, Manganese, Molybdenum, Copper, Iron and Zinc play vital role in plant the metabolism. Thiamine, pyridoxine, nicotinic acid act as enzymatic cofactors in universal pathways including glycolysis and TCA cycle along with primary and secondary metabolism in the plants. Glycine serves as a source of amino acid. Kinetin helps in shooting and IAA promotes callus formation and rooting.

The product is plant tissue culture tested but it is the sole responsibility of the user to ensure the suitability of the medium for individual species.

Composition**

Ingredients	mg/Litre
MACROELEMENTS	
Ammonium nitrate	1650.000
Calcium chloride	332.200
Magnesium sulphate	180.690
Potassium nitrate	1900.000
Potassium phosphate monobasic	170.000
MICROELEMENTS	
Boric acid	6.200
Cobalt chloride hexahydrate	0.025
Copper sulphate pentahydrate	0.025
EDTA disodium salt dihydrate	37.300
Ferrous sulphate heptahydrate	27.800
Manganese sulphate monohydrate	16.900
Molybdic acid (sodium salt)	0.213
Potassium Iodide	0.830
Zinc sulphate heptahydrate	8.600
VITAMINS	
Nicotinic acid (free acid)	0.500
Pyridoxine HCl	0.500
Thiamine hydrochloride	0.400
myo-Inositol	100.000
AMINO ACID	
Glycine	2.000
OTHERS	
Casein hydrolysate	1000.000
Indole-3-acetic acid (IAA)	2.000
Kinetin	0.200
Total (gms/litre)	5.4

Material required but not provided

- Autoclaved distilled water
- Plant growth regulators
- 1N NaOH/HCl
- Sucrose (PCT1607)
- Gelling agents like Agar (PCT1901) or CleriGel (PCT1903)

Quality Control

Appearance
White to off-white, homogenous, free flowing powder
Solubility
5.4 gms/litre soluble in distilled water
Colour and Clarity
Colourless to light yellow, clear solution
pH at 25ºC
5.10 - 6.10
Plant Tissue Culture Test
The growth promoting properties of medium is assessed by providing plant cultures with relative humidity of about 60%±2%, temperature 22ºC±2ºC and photoperiod of about 16:8. The plant species showed actively growing callus and shoots with no structural, necrotic and toxic deformity.

Directions

- Reconstitute medium by adding required quantity of powder in two-third of total volume with constant, gentle stirring till the medium gets completely dissolved.
- Add heat stable supplements prior to autoclaving.
- Make up the final volume with distilled water.
- Adjust the pH of the medium to 5.75 ± 0.5 using 1N NaOH/HCl.
- Add gelling agent and heat the medium to boiling till complete dissolution of gelling agent.
- Sterilize the medium by autoclaving at 15 lbs and 121°Cfor 15 min.
- Cool the autoclaved medium to about 45°C before adding heat labile supplements.
- Aseptically dispense the desired amount of medium under a laminar airflow unit in sterile culture vessels

Storage and Shelf Life

- The plant tissue culture medium powder is extremely hygroscopic and must be stored at 2-8°C in air tight containers.
- Preferably, entire content of each package should be used immediately after opening.
- Use before the expiry date.

Precautions

- Ensure appropriate pH of the medium before addition of gelling agent as acidic pH will lead to decreased gelation resulting in semi solid flowing gel while alkaline pH will lead to formation of hardened gel.
- Use of Distilled water/Tissue culture grade water is recommended for media preparation as tap water or lower grade water may lead to salt precipitation and improper gelation.
- Avoid preparation of concentrated solutions, as it will lead to precipitation of salts.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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