Technical Information

NLN Medium

With Vitamins And Without Calcium nitrate, Sucrose, Agar

Product Code: PT1094

Application: NLN Medium has been developed by Lichter in 1982 for the *in vitro* anther culture of *Brassica napus*, family *Brassicaceae*. The composition of NLN medium was derived from Nitsch medium. It is widely used to support the initiation and growth of haploid plants from anther and pollen cultures of *Brassica* species.

NLN Medium is a nutrient blend of inorganic salts, vitamins and amino acids. Potassium nitrate serves as a nitrogen source. Potassium dihydrogen phosphate serves as a phosphate source and enhances morphogenesis. Microelements like Boron, Manganese, Molybdenum, Copper, Iron and Zinc play vital role in plant metabolism and improves callus quality. Vitamins like folic acid acts as coenzyme while thiamine, pyridoxine, nicotinic acid, inositol and biotin act as enzymatic cofactors in the universal pathways including glycolysis and TCA cycle along with the primary and secondary metabolism in plants. Amino acids serine, glutamine glycine and glutathione provides reduced organic nitrogen and enhance formation of anther and pollen callus which redifferentiates to form haploids.

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	
Magnesium sulphate	61.043
Potassium nitrate	125.000
Potassium phosphate monobasic	125.000
MICROELEMENTS	
Boric acid	10.000
Cobalt chloride hexahydrate	0.025
Copper sulphate pentahydrate	0.025
EDTA disodium salt dihydrate	37.300
Ferrous sulphate heptahydrate	27.800
Manganese sulphate monohydrate	18.943
Molybdic acid (sodium salt)	0.213
Zinc sulphate heptahydrate	10.000
VITAMINS	
Folic acid	0.500
myo-Inositol	100.000
Nicotinic acid (free acid)	5.000
Pyridoxine HCl	0.500
Thiamine hydrochloride	0.500
D-biotin	0.050
AMINO ACID	
Glutathione	30.000
L-Glutamine	800.000
L-Serine	100.000
Glycine	2.000
Total (gms/litre)	1.5

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

1.5 gms/litre soluble in distilled water

Colour and Clarity

Colourless to light yellow, clear solution

pH at 25ºC

3.60 - 4.60

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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