

Technical Data

Gresshoff & Doy (DBM2) Vitamins Solution (1000X)

PL019

Composition:

Ingredients	milligrams/1ml
myo - Inositol	100.00
Thiamine hydrochloride	10.00
Pyridoxine hydrochloride	1.00
Nicotinic acid (Free acid)	1.00
Glycine (Free base)	4.00
TOTAL gm/1ml	0.12

Directions:

Measure out approximately 600ml of distilled water. While stirring the water, add 1ml of vitamins solution. Add desired heat stable supplements prior to autoclaving. Adjust the medium to the desired pH using 1N HCl/NaOH. Make up the final volume to 1000ml with distilled water. Sterilize the medium by autoclaving at 15 lbs or 121°C for 15 minutes. Cool the autoclaved medium to 45°C before adding the filter sterilized heat labile supplements. Dispense the desired amount of medium in sterile culture vessels.

Principle and Interpretation:

Gresshoff & Doy (DBM2) vitamins (1000X) is a filter sterilized stock solution. The stock contains vitamins and amino acid as described by Gresshoff & Doy (DBM2). Add 1ml per litre of vitamins stock solution to the prepared medium to achieve the desired concentration.

Quality Control:

Appearance : Colourless to yellow, clear solution. pH : 3.5 ± 0.5 of 50ml vitamins stock solution.

Sterility : No bacterial or fungal growth is observed after 14 days of

incubation, as per USP specification.

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Cultural Response:

Cultural condition:

• Incubation period : 5 weeks • Relative humidity : $60\% \pm 2\%$ • Temperature : $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ • Photoperiod (D: N) in hours : 16:8

Cell Line	Type of Culture	Results
Musa species	Shoot culture	No structural deformity observed
		No necrotic tissues,
		Actively growing shoots,
		No toxicity to shoots
Daucus species	Callus culture	No necrotic tissues,
		Actively growing callus,
		No toxicity to callus

[The medium is prepared as per direction. The growth promoting activity of this vitamins stock solution is evaluated using two plant species viz. *Musa* species and *Daucus* species through three passages. Plant growth hormones (e.g. 2,4-D, NAA, Kinetin and 6-BAP) are added in suitable combinations and concentrations.]

Storage and shelf life:

Store the vitamins stock solution at 2-8°C away from direct light. If possible, the content of each bottle should be used aseptically after opening and the unused portion should be stored at 2-8°C. Avoid contaminating the liquid. Use before the expiry date.

Reference:

1. Gresshoff P.M. & Doy C.H., Z. Pflanzenphysiol., (1974), 73, 132 - 141

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