Nitsch Medium **PT012**
w/ Vitamins and Sucrose;
w/o $CaCl_2$ and Agar

Composition :

Ingredients	milligrams/litre
Potassium nitrate	950.00
Ammonium nitrate	720.00
Magnesium sulphate anhydrous	90.34
Potassium phosphate monobasic	68.00
Manganese sulphate.H_2O	18.94
Boric acid	10.00
Molybdic acid (sodium salt).$2H_2O$	0.25
Zinc sulphate.$7H_2O$	10.00
Copper sulphate.$5H_2O$	0.025
Ferrous sulphate.$7H_2O$	27.85
EDTA disodium salt.$2H_2O$	37.25
myo - Inositol	100.00
Thiamine hydrochloride	0.50
Pyridoxine hydrochloride	0.50
Nicotinic acid (Free acid)	5.00
Folic acid	0.50
Biotin	0.05
Glycine (Free base)	2.00
Sucrose	20000.00
TOTAL gm/litre	**22.04**

Directions :

Suspend 22.02 grams of dehydrated medium# in 600ml of distilled water and rinse media vial with small quantity of distilled water to remove traces of powder. Apply constant gentle stirring to the solution till the powder dissolves completely. 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 aseptically in sterile culture vessels.

Weight after vacuum drying to remove all water

Principle and Interpretation :

Nitsch medium has been specially formulated for the *in vitro* culture of plant cell, tissue and organ culture. Ammonium nitrate and potassium nitrate serves as the sources of nitrate. Glycine serves as the source of amino acid. Sucrose serves as the carbohydrate source. Medium is devoid of calcium chloride and agar; hence these components have to be added to the medium prior to use.

Quality Control :

Appearance	: White to off-white, homogeneous, free flowing powder.
Solubility	: 22.02 gm/litre freely soluble in distilled water.
Colour and Clarity	: Colourless to light yellow, clear solution.
pH at 25°C	: 3.8 ±0.5 of 2.202% w/v dehydrated medium.

Cultural Response :

Cultural condition :

- Incubation period : 5 weeks
- Relative humidity : 60% ± 2%
- Temperature : 22°C ± 2°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 plant tissue culture medium 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 :

Dehydrated plant tissue culture media powder is extremely hygroscopic and should be protected from atmospheric moisture. If possible, the entire content of each bottle should be used immediately after opening or else the unused portion should be stored in a desiccator and refrigerated at 2-8°C. Use before the expiry date.

Reference :

1. Nitsch J.P. & Nitsch C., Science, (1969), 163, 85 - 87