Compositions:

**Ingredients** | **milligrams/litre**
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myo - Inositol | 100.00
Thiamine hydrochloride | 10.00
Pyridoxine hydrochloride | 1.00
Nicotinic acid (Free acid) | 1.00

**TOTAL gm/litre** | **0.11**

**Directions:**

Suspend 0.11 grams of dehydrated vitamins powder* in 600ml of distilled water. 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.

Principle and Interpretation:

De Greef & Jacobs / Gamborg B5 / Orchid vitamins (100X) powder is ready to use mixture of vitamins which is sufficient to prepare 100 litres of medium from content of one vial. The vial contains 11.20 gm of powdered vitamin mixture.

Quality Control :

- **Appearance**: White to off-white, homogeneous, free flowing powder.
- **Solubility**: 11.20 gm/litre soluble in distilled water.
- **Colour and Clarity**: Colourless to light yellow, clear solution.
- **pH at 25°C**: 3.3 ±0.5 of 1.12% w/v dehydrated powdered vitamins mixture.

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*Weight after vacuum drying to remove all water*
Cultural Response:

Cultural condition:
- Incubation period: 5 weeks
- Relative humidity: 60% ± 2%
- Temperature: 22°C ± 2°C
- Photoperiod (D:N) in hours: 16:8

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Type of Culture</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musa species</td>
<td>Shoot culture</td>
<td>No structural deformity observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No necrotic tissues,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Actively growing shoots,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No toxicity to shoots</td>
</tr>
<tr>
<td>Daucus species</td>
<td>Callus culture</td>
<td>No necrotic tissues,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Actively growing callus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No toxicity to callus</td>
</tr>
</tbody>
</table>

[The medium is prepared as per direction. The growth promoting activity of this vitamin mixture 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:

Powdered vitamin mixtures are very hygroscopic and must be protected from atmospheric moisture. It is advisable to use the entire contents of vial immediately after opening. In case of unused powder should be stored in desiccator and refrigerated at 2-8°C. Use before the expiry date.

Reference: