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# TECHNICAL INFORMATION

Catalog Number: 2623220, 2623222
**Murashige and Skoog basal salt medium with Gamborg's Vitamins**

**Physical Description:** White to off white powder

**Solubility:** Soluble in water (clear, colorless to slight yellow solution)

**Formulation:**

| Component | Quantity (mg/L) | Concentration |
|---|---|---|
| Ammonium Nitrate | 1650 | 20.61 uM |
| Boric Acid | 6.2 | 100 uM |
| Calcium Chloride, Anhydrous | 332.2 | 2.99 mM |
| Cobalt Chloride• $6H_2O$ | 0.025 | 0.11 uM |
| Cupric Sulfate• $5H_2O$ | 0.025 | 0.1 uM |
| Na 2 -EDTA | 37.26 | 100 uM |
| Ferrous Sulfate• $7H_2O$ | 27.8 | 100 uM |
| Magnesium Sulfate | 180.7 | 1.5 mM |
| Manganese Sulfate• $H_2O$ | 16.9 | 100 uM |
| Molybdic Acid, Sodium Salt, $2H_2O$ | 0.25 | 1.03 uM |
| Potassium Iodide | 0.83 | 5 uM |
| Potassium Nitrate | 1900 | 18.79 mM |

| | | |
|---|---|---|
| Potassium Phosphate Monobasic | 170 | 1.25 mM |
| Zinc Sulfate• $7H_2O$ | 8.6 | 29.91 uM |
| myo-Inositol | 100 | 0.56 uM |
| Nicotinic Acid | 1 | 8.12 uM |
| Pyridoxine HCl | 1 | 4.86 uM |
| Thiamine | 10 | 29.65 uM |

**Preparation:** To prepare 1 liter, use 4.44 grams of powder

**pH:** 3.75-4.75

**Description:** Supports or facilitates plant growth and/or shoot proliferation in plant tissue cultures. Includes the macro- and micronutrients as described by Murashige and Skoog, 1962, and vitamins as described by Gamborg, et al., 1966.

**Preparation Instructions:**

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. If possible the entire contents of each package should be used immediately after opening. Preparing the medium in a concentrated form is not recommended as some salt added to the medium may affect shelf life and storage conditions. The basic steps for preparing the culture medium are listed below:

– Measure out approximately 90% of the final required volume of tissue culture grade water, e.g. 900 ml for a final volume of 1000 ml. Select a container twice the size of the final volume.
– While stirring the water add the powdered medium and stir until completely dissolved. Heating may be required to bring powders into solution.
– Rinse the original container with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in step 2.
– Add desired heat stable components (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.).
– Add additional tissue culture grade water to bring the medium to the final volume.
– While stirring, adjust medium to desired pH using NaOH, HCl or KOH.
– If a gelling agent is used, heat until the solution is clear.
– Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
– Sterilize the medium in a validated autoclave at 1 $kg/cm^2$ (15 psi), 121°C, for the desired time period.
– Allow media to cool prior to use.

***NOTE:*** Precipitates are known to occur, with time, in plant tissue culture media. The precipitates have been analyzed (Dalton, et al., 1983). They are composed of small, pale yellow-white particles. Analysis of precipitates indicated a predominance of iron, phosphate and zinc. The probable cause of the precipitates is the inevitable oxidation of ferrous ions. When the solubility of ferric phosphate occurs. There are no reports of detrimental effects on growth and development in plant tissue culture due to the precipitates.

**Sterilizing Nutrient Media**

Two methods (autoclaving and membrane filtration under positive pressure) are commonly used to sterilize culture media. Culture media, distilled water, and other stable mixtures can be autoclaved in glass containers that are sealed with cotton plugs, aluminum foil, or plastic closures. However, solutions that contain heat-labile components must be filter-sterilized.

Generally, nutrient media are autoclaved at 15 psi and 121°C. For small volumes of liquids (100 ml or less), the time required for autoclaving is 15-20 minutes, but for larger quantities (2-4 liter), 30-40 minutes is required. The pressure should not exceed 20 psi, as higher pressures may lead to the decomposition of carbohydrates and other thermolabile components of a medium.

Since many proteins, vitamins, amino acids, plant extracts, hormones, and carbohydrates are thermolabile and may decompose during autoclaving, filter sterilization may be required. A Millipore® or Seitz® filter can be used; the porosity of the filter membrane should be no larger than 0.2 microns. Empty glassware that is to hold media must be sterilized in an autoclave

before filter sterilization.

Nutrient media that contain thermolabile components can be prepared in several steps. That is, a solution of the heat-stable components is sterilized in the usual way by autoclaving, then cooled to 50-60°C under sterile conditions; in a separate operation, solutions of the thermolabile components are filter-sterilized. The sterilized solutions are then combined under aseptic conditions to give the complete media.

**Sterilizing Plant Material**

Obtaining sterile plant material is difficult, and despite any precautions taken, 95% of cultures will end up contaminated if the explant is not disinfected in some manner. Because living materials cannot be exposed to extreme heat and retain their biological capabilities, plant organs and tissues are sterilized by treatment with a disinfecting solution. Solutions used to sterilize explants must preserve the plant tissue but at the same time destroy any fungal or bacterial contaminants.

Once explants have been obtained, they should be washed in a mild soapy detergent before treatment with a sterilizing solution. Some herbaceous plant materials (e.g. African violet leaves) may not require this step, but woody material, tubers, etc., must be washed thoroughly. After the tissue is washed, it should be rinsed under running tap water for 10-30 minutes and then be submerged into the disinfectant under sterile conditions. All surfaces of the explant must be in contact with the sterilant. After the allotted time for sterilization, the sterilant should be decanted and the explants washed at least three times in sterile distilled water. For materials that are difficult to disinfect, it may be necessary to repeat the treatment 24-48 hours before making the final explants. This allows previously unkilled microbes time to develop to a stage at which they are vulnerable to the sterilant.

Availability:

| Catalog Number | Description | Size |
|---|---|---|
| 2623222<br>2623220 | Murashige and Skoog Basal Medium with Gamborg's Vitamins | 1 x 10 liter<br>10 x 1 liter |

Also Available:

| Catalog Number | Description | Size |
|---|---|---|
| 100262 | Agar, 80-100 mesh | 100 g<br>250 g<br>500 g<br>1 kg |
| 100266 | Agar, Shredded | 100 g<br>250 g<br>500 g |
| 150178 | Agar, Bacteriological Grade | 100 g<br>250 g<br>500 g |
| 150180 | Gel-Gro, Agar replacement | 100 g<br>250 g<br>500 g<br>1 kg |

**References:**


– Murashige, T., Personal Communication.
– Murashige, T., and Skoog, P., *Physiol. Plant*, **v. 15**, 473-97 (1962).
– Gamborg, O.L., *Plant Physiol.*, **v. 45**, 372 (1970).