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Way of Life through  
Plants™

### PLANT TISSUE CULTURE MEDIA PREPARATION

Formulation charts posted on the "Technical Literature" page on web site

#### FROM PACKED POWDER

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:

1. 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.
2. While stirring the water add the powdered medium and stir until completely dissolved.
3. 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.
4. Add desired heat stable supplements (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.)
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH, HCl, or KOH.
7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm<sup>2</sup> (15 psi), 121°C, for the time period described under Sterilization of Media.
10. Allow medium to cool prior to use. \*Heating may be required to bring powders into solution.

**POWDERED MEDIA AND BASAL SALT MIXTURES ARE FOR LABORATORY USE ONLY.  
NOT FOR DRUGS, HOUSEHOLD OR OTHER USES.**

#### MATERIALS NOT PROVIDED

Deionized tissue culture grade water

1 N Hydrochloric Acid (HCl) (Product No. H 245)

1 N Sodium Hydroxide (NaOH) (Product No. S 835)

1 N Potassium Hydroxide (KOH) (Product No. P 682)

Auxins, cytokinins, carbohydrates, gelling agents, and other supplements listed in the Biochemical's section.

**PhytoTechnology Laboratories, Inc.**

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Web Site: [www.phytotechlab.com](http://www.phytotechlab.com)

## STORAGE

Store dry medium in at 0-5°C. Deterioration of powdered medium may be recognized by: 1) color variations; 2) granulation, clumping, or particulate matter throughout the powder; 3) insolubility; 4) pH change; or 5) inability to promote growth when properly used.

## PRECIPITATION IN PLANT TISSUE CULTURE POWDERED MEDIA

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.

## PREPARATION FROM BASAL SALT SOLUTIONS

Liquid 10X solutions are offered for your convenience. To avoid precipitation over long-term storage, *Phyto*Technology Laboratories has formulated two solutions which when mixed at the proper dilution make a solution with the appropriate salt concentration. The basic steps for preparing 1 liter of culture medium are listed below.

**CAUTION:** Do not autoclave product in bottle. The bottle is **NOT** autoclavable.

1. Measure out approximately 700 ml of tissue culture grade water.
2. While stirring the water, add 100 ml of Macronutrient Solution (Product No. M 654)
3. Continue stirring the mixture while adding 100 ml of Micronutrient Solution (Product No. M 529)
4. Add desired heat stable supplements (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.)
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH, HCl or KOH.
7. If gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm<sup>2</sup> (15 psi), 121°C, for the time period described under Sterilization of Media.
10. Allow medium to cool prior to use.

**BASAL SALT SOLUTIONS ARE FOR LABORATORY USE ONLY. NOT FOR DRUG, HOUSEHOLD, OR OTHER USES.**

## MATERIALS NOT PROVIDED

Deionized tissue culture grade water

1 N Hydrochloric Acid (Product No. H 245)

1 N Sodium Hydroxide (Product No. S 835)

1 N Potassium Hydroxide (KOH) (Product No. P 682)

Medium additives as required

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## STORAGE

Store basal salt at 0-5°C. Deterioration of basal salt solutions may be recognized by: 1) color change; 2) pH change; 3) precipitation of components; or 4) inability to promote growth when properly used.

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## VITAMIN PREPARATION AND USE

### VITAMIN MIXTURES

Powdered vitamin mixtures are hygroscopic and must be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. The basic steps for preparing 1000X concentrated solutions with vitamin mixtures are listed below:

1. Measure out 70% of the final required volume of deionized-distilled water (e.g. 70 ml for a final volume of 100 ml)
2. While stirring the water add the powdered vitamin mixture. Stir until completely dissolved. Increasing the pH and/or warming solution (35-37°C) may be required.
3. Rinse the original container with a small volume of water to remove traces of the powder. Add to the solution in Step No. 2.
4. Add additional water to bring the medium to the final volume.
5. The resulting 1000X concentrated solution should be used at a concentration of 1 ml/L of medium.
6. Follow the same steps to prepare a 100X concentrated solution and use at 10 ml/L of medium.

### VITAMIN SOLUTIONS

1. The vitamin solutions are sterile filtered through a double 0.2 µm filtration unit and are ready for use.
2. Vitamin solutions should be added at a concentration of 1 ml/L of medium to prepare the final recommended concentration of vitamins in the medium.

### MATERIALS NOT PROVIDED

#### BASAL MEDIA AND ADDITIVES AS REQUIRED

Deionized tissue culture grade water

1 N Hydrochloric Acid (Product No. H 245)

1 N Sodium Hydroxide (Product No. S 835)

1 N Potassium Hydroxide (KOH) (Product No. P 682)

**VITAMIN MIXTURES AND SOLUTIONS ARE FOR LABORATORY USE ONLY. NOT FOR DRUG, HOUSEHOLD, OR OTHER USES.**

### STORAGE

Store vitamin mixtures and solutions in a refrigerator at 0-5°C. Precipitation in vitamin solutions may occur during storage. This can be redissolved by warming the solution in a water bath (35-37°C) for a short period of time.

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