



Preparing Stock Solutions

The use of stock solutions reduces the number of repetitive operations involved in media preparation and, hence, the chance of human or experimental error. Moreover direct weighing of media components (e.g., micronutrients and hormones) that are required only in milligram or microgram quantities in the final formulation cannot be performed with sufficient accuracy for tissue culture work. For these components, preparation of concentrated stock solutions and subsequent dilution into the final media is standard procedure. In addition, concentrated solutions of some materials are more stable and can be stored for longer periods than more dilute solutions.

To prepare a stock solution, weigh out the required amount of the compound and place it in a clean flask. It is common practice to make a stock solution 10x or 100x, depending upon the solubility of the compound. Once the chemical is in the flask, dissolve it in a small amount of water, ethyl alcohol, 1 N NaOH, or 1 N HCL. Next, slowly add double-distilled water to the flask, while agitating. Continue this until the proper volume is reached. Label the flask with the name of the solution, preparation and expiration dates, and the name of the person who prepared the solution. Certain items, e.g., IAA, must be prepared and stored in amber bottles to prevent photodecomposition. The volumes of stock solutions prepared at various concentrations that must be used to achieve various final concentrations are presented in tabular form in the Plant Growth Regulator Section.

Macronutrients

Stock solutions of macronutrients can be prepared at 10 times the concentration of the final medium. A separate stock solution for calcium salts may be required to prevent precipitation. Stock solution of macronutrients can be stored safely for several weeks in a refrigerator at 2°-4°C.

Micronutrients

Micronutrient stock solutions are generally made up at 100 times their final strength. It is recommended that micronutrient stocks be stored in either a refrigerator or freezer until needed. Micronutrient stock solutions could be stored in a refrigerator for up to 1 year without appreciable deterioration. Iron stock solutions should be prepared and stored separately from other micronutrients in an amber storage bottle. Formulations for preparing stock solutions of iron are presented later.

Vitamins

Vitamins are prepared as 100X or 1000X stock solutions and stored in a freezer (-20°C) until used. Vitamin stock solutions should be made up each time media is prepared if a refrigerator or freezer is not available. Vitamin stock solutions can be stored safely in a refrigerator for 2-3 months but should be discarded after that time.

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Growth Regulators

The auxins NAA and 2,4-D are considered to be stable and can be stored at 4°C for several months; IAA should be stored at -20°C. Auxin stock solutions are generally prepared at 100-1000 times the final desired concentrations. Solution of NAA and 2,4-D can be stored for several months in a refrigerator or indefinitely at -20°C. Generally IAA and 2,4-D are dissolved in a small volume of 95% ethyl alcohol or KOH and then brought to volume with double-distilled water; NAA can be dissolved in a small amount of 1 N NaOH or KOH, which also can be used to dissolve 2,4-D and IAA.

The cytokinins are considered to be stable and can be stored at -20°C. Cytokinin stock solutions are generally prepared at 100X to 1000X concentrations. Many of the cytokinins are difficult to dissolve, and a few drops of either 1 N HCL, 1 N NaOH, in KOH or DMSO, are required to bring them into solution.

Storage of Stock Solutions

Storage conditions for most stock solutions have already been pointed out; however, some additional points can be made. For convenience, many labs prepare stock solutions and then divide them into aliquots sufficient to prepare from 1 to 10 liter of medium; these aliquots are stored in small vials or plastic bags in a freezer. This procedure removes the inconvenience of having to un-thaw a large volume of frozen stock each time medium is prepared. Some have found that heating in a microwave oven is a satisfactory and quick method of thawing concentrated medium.

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