

## PREPARATION OF LABORATORY REAGENTS FOR AGRICULTURAL SCIENCE

### ***Agar.***

Mix 1.5 g of agar powder [+selected nutrient] to 100 mls water in a conical flask. Stir constantly and bring to the boil. Plug the top of the flask with cotton wool and cover with aluminium foil. Place in an oven at 120<sup>0</sup>C for 15 mins.

### ***Aluminium Chloride:***

Dissolve 4.82g aluminium chloride in distilled water and make up to 1 Litre.

### ***Ammonia:***

Use a 10% Solution. Dilute 10cm<sup>3</sup> of ammonia to 100cm<sup>3</sup> of water in a fume cabinet.

### ***Ammonium Molybdate:***

Dissolve 5g Ammonium Molybdate in 50cm<sup>3</sup> of distilled water.

### ***Ammonium Oxalate:***

Dissolve 20g Ammonium oxalate in distilled water and make up to 1 Litre.

### ***Amyl Alcohol: Commercially Purchase***

#### ***Amylase solution;***

Dissolve 1 g of amylase in 100 mls of de-ionised water. This solution does not keep well and should be made up freshly when required.

#### ***Ascorbic Acid: Vitamin C.- Commercially Purchase***

#### ***Barium Chloride:***

Dissolve 122g barium chloride in distilled water and make up to 1 Litre.

#### ***Benedict's solution\* - Commercially Purchase***

Dissolve 17.3 g of hydrates copper (II) sulphate crystals in 200 cm<sup>3</sup> deionised water. In a separate container, dissolve 100 g of anhydrous sodium carbonate and 173 g of sodium citrate in 700 cm<sup>3</sup> deionised water. Add the copper sulphate solution to the sodium carbonate/sodium citrate solution, stirring continuously. Make up the solution to 1 litre.

#### ***Biuret solution -Commercially Purchase***

Biuret solution can be purchased from suppliers. Dilute as per manufacturer's instructions.

#### ***Calcium Chloride solution***

Grind 1.4 g of dry calcium chloride using a pestle and mortar; add to 100 mls water.

#### ***Calcium hydroxide solution (limewater)***

This is usually made up as a saturated solution. The solubility of calcium hydroxide is only about 1.5 g/L. Place about 100 g in a 2.5-litre Winchester bottle. Add about 2 litres

of water and shake well. Allow the solid to settle and decant the clear limewater when required. Keep the bottle topped up with water. Use a rubber stopper to protect the limewater from atmospheric carbon dioxide. It is a good idea to test the limewater before use by blowing into a sample of it in a test tube using a drinking straw.

***Chloroform: Commercially Purchase***

***Cobalt chloride paper \*- Commercially Purchase.*** (The paper may be dried using a warm oven, a warm radiator or a hair dryer.)

***Copper sulphate solution (0.25 M)***

Add 1g copper sulphate to 100cm<sup>3</sup> distilled water.

***DCPIP- Tablet Form-Follow Manufacturers Instructions***

***Diphenylamine:*** Dissolve 0.2g of diphenylamine in 100cm<sup>3</sup> of Concentrated Sulphuric Acid (handle with care. Use a fume cabinet)

***Ethanol- Commercially Purchase (Industrial Methylated spirits)***

***Fehling's solution***

Fehling's solution is made up by mixing equal quantities of Fehling's solution I and Fehling's solution II. The mixture does not keep and it should be made up only when required. The resulting mixture should be a clear royal blue colour. Fehling's solution I and Fehling's solution II are made up as follows:

***Fehling's solution I\*:*** Dissolve 63 g of copper sulphate crystals in distilled water and make up to 1 litre. If the solution is cloudy add 1 cm<sup>3</sup> of concentrated sulphuric acid.

***Fehling's solution II\*:*** Dissolve 352 g of potassium sodium tartrate (Rochelle Salt) and 154 g of sodium hydroxide in water and make the solution up to 1 litre.

***Glucose solution (1%)***

Dissolve 1 g glucose powder in 100 cm<sup>3</sup> water.

***Glucose solution (5%)***

Dissolve 5 g glucose powder in 100 cm<sup>3</sup> still warm, boiled water. Do not shake. Store in a stoppered bottle.

***Hydrochloric Acid (0.1M)***

Carefully add 8.6cm<sup>3</sup> concentrated hydrochloric acid in distilled water and make up to 1 Litre in a fume cabinet.

***Hydrochloric Acid Dilute (1M)***

Carefully add 86cm<sup>3</sup> concentrated hydrochloric acid in distilled water and make up to 1 Litre in a fume cabinet.

***Hydrogen peroxide (100 volumes)- Commercially Purchase (20 vol)***

As purchased, hydrogen peroxide is normally of 100-volume strength, i.e. one volume of this solution will yield 100 volumes of oxygen when it is decomposed. It is a powerful oxidising agent and should be handled with great care. Always wear rubber gloves when using it, as it causes skin burns. For laboratory use (e.g. as a substrate for the enzyme catalase), 20-volume hydrogen peroxide is normally used. To make this up, add 200 cm<sup>3</sup> of 100-volume hydrogen peroxide to water and make it up to 1 litre with deionised water.

***Iodine solution***

Stock solution – Grind 1 g of iodine crystals and 1 g of potassium iodide crystals in a mortar while adding distilled water. Pour the solution into a graduated cylinder and dilute to 100 cm<sup>3</sup>. This solution should be stored in a brown glass bottle.

**Dilute iodine solution** or commercially purchase.

Add 5 cm<sup>3</sup> of the stock solution to 100 cm<sup>3</sup> distilled water. This solution does not keep well.

***Iodine stain\*- Commercially purchase***

Use stock solution as described above.

***Limewater, see Calcium hydroxide solution***

***Methylene blue stain\*- Commercially Purchase***

Dissolve 1 g methylene blue and 0.6 g of NaCl in 100 cm<sup>3</sup> distilled water.

***Methylated Spirits: Commercially Purchase***

***Potassium Chloride (1%)***

Dissolve 1g of potassium chloride in distilled water and make up to 100cm<sup>3</sup>.

***Resazurin Tablets: Follow manufacturers instructions***

***Sach's Water Culture Solutions: As per manufacturer's instructions.***

***Silver Nitrate:***

Dissolve 17g silver nitrate in distilled water and make up to 1 litre. Store in brown bottle away from light.

***Sodium Chloride:***

Dissolve 5.85g sodium chloride (salt) in distilled water and make up to 1 Litre.

***Sodium hydrogen carbonate, saturated solution***

Dissolve 10 g sodium hydrogen carbonate (Sodium bicarbonate or bread soda) in 100 cm<sup>3</sup> water to give a saturated solution. Vary the concentrations of sodium hydrogen carbonate solutions by dilution with water.

### ***Sodium hydroxide solution (2M)***

Carefully dissolve 80 g NaOH pellets in 1 litre of cold water. A great deal of heat is evolved and it is helpful to have the beaker standing in water in a sink. Stir the solution continually while adding the pellets and continue stirring until all the pellets have dissolved.

### ***Sodium pyrogallol, alkaline***

Place 100 cm<sup>3</sup> of 2M sodium hydroxide solution in a beaker and add a few crystals of pyrogallic acid. The solution turns brown.

### ***Starch solution (2%)***

Weigh out 2 g starch powder. Add to a small amount of cold water in a beaker and make a slurry. Then make up to 100 cm<sup>3</sup> with boiling water, stirring constantly. Use a good quality starch, e.g. Analar. This starch solution does not keep well and should be made up fresh on the required day.

### ***Sucrose: (10%)***

Dissolve 10g sucrose (table sugar) in 100cm<sup>3</sup>.

### ***Sulphuric acid, concentrated\*- Commercially Purchase***

As purchased, the acid is 18M and quite dense. Take care when handling it, as a full Winchester is very heavy. Remember never lift a bottle by the cap and always support a heavy bottle underneath. The concentrated acid is extremely dangerous and highly corrosive. Great care must be taken when using it.

### ***Sulphuric acid, dilute***

NEVER ADD WATER TO CONCENTRATED SULPHURIC ACID! Always add acid to the water. Since considerable heat is generated when concentrated sulphuric acid is added to water, the dilution should be carried out in a large Pyrex beaker standing in a sink of cold water. Do not use a Winchester bottle for the dilution, as the heat generated has been known to crack the thick glass.

Place 750 cm<sup>3</sup> deionised water into a large Pyrex beaker standing in a basin or sink containing cold water.

Slowly and carefully add 55 cm<sup>3</sup> concentrated sulphuric acid to the water in the beaker, stirring constantly. Make the solution up to 1 litre with deionised water when cool.