

Date:

Name:

Topic:                    **Preparation of 1 L of Tissue Culture medium**

1. Weigh 6.5 g of agar and put it into 250 ml deionised or distilled water in SIMAX bottle, stir and melt it in autoclave.
2. Put 500 ml of deionised or distilled water into an Erlenmeyer bottle.
3. Add the appropriate amount of the macro elements stocks (100 ml), micro elements stocks (10 ml) and chelated iron stock (5 ml).
4. Add the vitamins (1 ml freeze concentrate).
5. Weigh 100 mg of inositol.
6. Weigh 20 g of sucrose.
7. Add plant growth regulators and other components like charcoal, if necessary.
8. Adjust the pH using 0.1 M KOH or 0.1 M HCl to 5.7.
9. Add melted agar; adjust the final volume to 1L with warmed distilled water.
10. Thoroughly mix and distribute the medium into the tissue culture vessels (40 ml into each vessel).
11. Cap the vessels.
12. Autoclave for 20 min at 121°C. If a large quantity is being autoclaved, longer times may be necessary.
13. For short-term storage keep media at room temperature, for prolonged storage keep media in the refrigerator.

Note:

In case when we use commercial mixture for medium preparation we use amount of those mixtures according to producer information.