

➤ **GENERAL MEDIUM FOR ROUTINE CULTIVATION OF BACTERIA**

PREPARATION OF NUTRIENT SUCROSE AGAR (NSA) MEDIA

Material Required

Peptone(5g), beef extract (3g), sucrose(20g), agar(20g),one-liter beakers(2),250-ml Erlenmeyer flasks (5), muslin cloth/cheese cloth, deionized water, measuring cylinder (1 L), non-absorbent cotton, heating arrangement.

Procedure

Take 500 ml of water in a one-liter sauce pan. Add 5g of peptone and 3g of beef extract to the water in the sauce pan and boil till they dissolve. Filter the nutrient extract through cheese cloth. Add 20 g of sucrose to the above nutrient extract. Take 500 ml of water in another beaker and heat it. Add 20g agar bit by bit to the hot water (96°C) to dissolve it. Mix the agar with the nutrient extract. Bring the volume upto 1000ml with the addition of distilled water. Dispense 200ml each to five conical flasks or dispense 8 ml each in screw-capped tubes/test tubes when slants or deep tubes are to be prepared. Plug the flasks and test tubes containing the medium. Sterilize at 121°C and 15 pounds of pressure for 15 minutes in an autoclave. Allow the tubes to cool in slanting position (for agar slants) and upright position (for agar deep tubes). Allow the flasks to cool until the flasks can be held by hand. Pour the medium into Petri dishes quickly under aseptic conditions. Allow the medium to gel to produce agar plates. The sterilized medium (in flasks or tubes) can be stored at room temperature (25°C) in a dust-free environment (if to be used within a week) or in a refrigerator (if to be stored longer).

Precautions

Do not fill the medium in excess of two-thirds of the capacity of the flasks/container used for autoclaving. Cotton plugs should be kept loose when autoclaving. Do not pour the media into Petri plates that are too hot since this will produce much condensed water on the Petri plate lid and thus can fall onto the agar surface and may lead to culture contamination. Pour the medium quickly to avoid contamination by air spores and close the lid as soon as possible. Perform the pouring of the medium in a cabinet fitted with UV tubes or in laminar-filtered air flow. Pouring is to be performed near the flame or under aseptic conditions. Medium containing slants or deep tubes are to be stored always at low temperature in dust-free environments.