

Supplementing TSB with agar is useful for the cultivation of anaerobes. The agar in the medium retards the absorption of oxygen by reducing convection currents in the medium.

Procedure

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours. An efficient and easy way to obtain suitable anaerobic conditions is through the use of BBL™ GasPak™ EZ anaerobic systems or an alternative anaerobic system.⁴

The organisms to be cultivated must first be isolated in pure culture on an appropriate plated or slanted medium.

Using a sterile inoculating loop or needle, transfer fresh growth from the plate or slant to the tubed medium to achieve the desired concentration of viable organisms. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Tubed media intended for isolation and cultivation of anaerobes should be incubated under anaerobic conditions for up to 7 days.

Expected Results

Growth in tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by Gram staining and subculturing onto appropriate media; e.g., a Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plate and/or Chocolate II Agar plate, EMB Agar or MacConkey II Agar plate, etc. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a GasPak EZ anaerobic system.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Fredette, Auger and Forget. 1961. Can. J. Med. Assoc. J. 84:164.
3. Forbes, Sahm, and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
4. Seip and Evans. 1980. J. Clin. Microbiol. 11:226.

Availability

BBL™ Trypticase™ Soy Broth with 0.15% Agar

Cat. No. 298263 Prepared Tubes (K Tubes), 9 mL – Ctn. of 100

Trypticase™ Soy Broth with 20% Glycerol

Intended Use

This medium is used in the long-term frozen maintenance of bacterial stock cultures.

Summary and Explanation

Trypticase Soy Broth, a nutritious medium supplemented with glycerol, may be used as a maintenance medium for the preservation of bacterial cultures.^{1,2}

Principles of the Procedure

Enzymatic digests of protein substrates act as protective colloids. Glycerol is a cryoprotective agent that provides intracellular and extracellular protection against freezing.²

Procedure

Using a sterile swab or inoculating loop, remove fresh growth from the plated or slanted medium and suspend in the broth maintenance medium to achieve the desired concentration of viable cells. Freeze suspension immediately at -20°C or below. Consult texts for detailed information about preparing stock cultures of specific organisms.²⁻⁴

Expected Results

Bacterial stock cultures frozen and stored at -20°C or below will remain viable for several months, and some may remain viable for years.

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Gherna. 1994. In Gerhardt, Murray, Wood and Krieg (ed.). Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
3. Norris and Ribbons (ed.). 1970. Methods for microbiology, vol. 3A. Academic Press, Inc., New York, N.Y.
4. Kirsop and Snell (ed.). 1984. Maintenance of microorganisms. Academic Press, Inc., New York, N.Y.

Availability

BBL™ Trypticase™ Soy Broth with 20% Glycerol

Cat. No. 296346 Prepared Tubes (K Tubes), 1.5 mL – Pkg. of 10
297808 Prepared Tubes (K Tubes), 1.5 mL – Ctn. of 100
297352 Prepared Tubes (C Tubes), 10 mL – Ctn. of 100

Tryptone • Trypticase™ Peptone

(See Casitone)