Technical Information

Anaerobic Agar

Product Code: DM 1228

Application: - Agar is recommended for the cultivation of anaerobic bacteria, especially *Clostridium* species and other anaerobic organisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Dextrose	10.000
Sodium chloride	5.000
Sodium thioglycollate	2.000
Sodium formaldehyde sulphoxylate	1.000
Methylene blue	0.002
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Anaerobic Agar was originally designed by Brewer [ii] for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates. This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology which can be readily seen on the light coloured agar medium [2,3]. Further Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements [3]. As there lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor [4].

This medium contains sodium thioglycollate and sodium formaldehyde sulphoxylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which gives blue colour to medium in presence of oxygen. Casein enzymic hydrolysate and dextrose provide essential nutrients while sodium chloride maintains osmotic equilibrium.

Pour 50-60 ml medium per 95 x 20 mm plate. For best results, use porous tops for the plates during solidification to get the dry surface. Inoculation can be done by streaking or smearing. Cover the inoculated plate with sterile Brewer Anaerobic Petri dish cover and Incubate aerobically, When standard plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium. After solidification, incubate anaerobically as per requirement of particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

Methodology

Suspend 58 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range:- 7.00-7.40

Cultural Response/Characteristics

DM 1228: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Clostridium butyricum ATCC 13732	50-100	good-luxuriant	>=50%
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Brewer J. H., 1942, Science, 95:587.
2. Vera J., 1942, J. Bacteriol., 44:497.
3. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
4. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis, Mo.

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