



## Anaerobic Agar

M228

Anaerobic 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 sulphonylate	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

### Directions

Suspend 58 grams in 1000 ml distilled water. Heat to boiling 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.

### Principle And Interpretation

Anaerobic Agar was originally designed for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates (1). This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible (2,3). Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements (3). Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor (4).

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

Dispense 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. Incubate aerobically, as desired. 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 required by particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

### Quality Control

#### 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

7.00-7.40

#### Cultural Response

M228: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

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Organism	Inoculum (CFU)	Growth	Recovery
<b>Cultural Response</b>			
<i>Clostridium butyricum</i> ATCC 13732	50-100	good-luxuriant	>=50%
<i>Clostridium perfringens</i> ATCC 12924	50-100	good-luxuriant	>=50%
<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant	>=50%

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on label.

### Reference

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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