

## Anaerobic HiVeg™ Agar

MV228

### Intended Use:

Recommended for the cultivation of anaerobic bacteria, especially *Clostridium* species and other anaerobic organisms.

### Composition\*\*

Ingredients	g / L
HiVeg™ hydrolysate	20.000
Dextrose (Glucose)	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 58.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Anaerobic HiVeg™ Agar is prepared by using HiVeg™ hydrolysate instead of tryptone which makes the medium BSE/TSE risks free. Anaerobic HiVeg™ Agar is the modification of Anaerobic Agar that was originally designed for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms in 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 (2). 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 sulphonylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen. HiVeg™ hydrolysate provides nitrogen and carbon compounds, long chain amino acids, vitamins and essential nutrients. Dextrose is the energy source. 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.

### Type of specimen

Please add specimens

### Specimen Collection and Handling

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Ensure that the clinical samples are properly transported under anaerobic conditions.
2. Proper anaerobic conditions must be maintained for optimal recovery of organisms
3. Methylene blue is toxic to certain anaerobes

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## 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

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

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

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20- 30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,5).

## Reference

1. Brewer J. H., 1942, Science, 95:587.
2. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
3. Vera J., 1942, J. Bacteriol., 44:497.
4. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis, Mo.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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