



## Antibiotic HiVeg Assay Medium No.1 (Seed HiVeg Agar)

MV003

Antibiotic HiVeg Assay Medium No.1 (Seed HiVeg Agar) is used for the microbiological assay of Beta-lactam and other antibiotics.

### Composition\*\*

Ingredients	Gms / Litre
HiVeg peptone	6.000
HiVeg hydrolysate	4.000
Yeast extract	3.000
HiVeg extract	1.500
Dextrose	1.000
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 30.5 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.

*Advice : Recommended for the microbiological assay of Bacitracin, Cephalexin, Cephaloglycin, Cephadrine, Cephaloridine, Cephalothin, Cephaperin, Cephazolin, Cloxacillin Cycloserine, Dicloxacillin, Methicillin, Nafcillin, Novobiocin, Oxacillin, Penicillin-G and Phenoxymethyl Penicillin .*

### Principle And Interpretation

Antibiotic HiVeg Assay Medium No.1 (Seed HiVeg Agar) is prepared by replacing animal based peptones with vegetable peptones, making the medium BSE-TSE risks free. It can be used for the same purpose of Antibiotic Assay Medium No.1 (Seed Agar). The potency of an antibiotic can be determined by chemical, physical and biological assays. Biological assays offer the most convenient method (1), since a reduction in the antimicrobial activity of a specific antibiotic is not usually displayed in chemical methods (2). Biological testing may be performed by either dilution (turbidimetric) or diffusion methods. The choice of methodology is often based on many factors, including relative ease of performance, flexibility and use of automated or semi-automated devices for both identification and susceptibility testing (3). Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (4). Antibiotic HiVeg Assay Medium No.1 is used in the microbiological assay of  $\beta$ -lactam and other antibiotics and as seed agar with *Micrococcus luteus* (ATCC 9341) for plate assay of Bacitracin, with *Staphylococcus aureus* (ATCC 29739) for cylinder plate assay of Cephalexin, Cephalothin, Cephaperin, Cloxacillin, Dicloxacillin, Methicillin, Nafcillin, Oxacillin, Penicillin-G and *Staphylococcus epidermidis* (ATCC 12228) for plate assay of Novobiocin. This media can be used according to the specifications detailed in various pharmacopoeias (2,5,6) and by the FDA (7).

Antibiotic assays are normally performed in freshly prepared media. Test organisms are spread evenly over the surface of solidified base agar. After incubation, the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results.

Nutrients and growth factors are supplied by the HiVeg peptone, HiVeg hydrolysate, yeast extract and HiVeg extract. Dextrose is supplemented as a carbon and energy source.

*Note: For Antibiotic Assay Methods and Selection of Antibiotic HiVeg Assay Medias, Refer Section Antibiotic HiVeg Assay Media.*

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm,comparable with 1.5% Agar gel

**Colour and Clarity of Prepared medium**

Yellow coloured clear to slightly opalescent gel forms in Petriplates.

**Reaction**

Reaction of 3.05% w/v aqueous solution at 25°C. pH : 6.6±0.2

**pH**

6.40-6.80

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

**Cultural Response**

Organism	Inoculum (CFU)	Growth	Recovery	Inoculum medium	Assay medium Inoculum & Assay medium
<b>Cultural Response</b> <i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	>70%	Framycetin, Josamycin, propionate, Kanamycin B, Spiramycin, Streptomycin	Streptomycin Josamycin
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	luxuriant	>50%	Colistimethate sodium, Colistin, Polymyxin B	
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	>70%	Chloramphenicol	
<i>Bacillus cereus</i> var <i>mycoides</i> ATCC 11778	50-100	luxuriant	>70%	Oxytetracycline, Tetracycline	
<i>Bacillus pumilis</i> ATCC 14884	50-100	luxuriant	>70%	Chlortetracycline, Framycetin, Kanamycin sulphate	
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	>70%	Capreomycin, Dihydrostreptomycin, Neomycin, Streptomycin, Troleandomycin	
<i>Micrococcus luteus</i> ATCC 9341	50-100	luxuriant	>70%	Erythromycin, Rifamycin	
<i>Micrococcus luteus</i> ATCC 10240	50-100	luxuriant	>70%		Bacitracin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	>70%	Carbenicillin	
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	>70%	Amikacin, Cephalothin, Chlortetracycline, Nafcillin, Tetracycline, Tobramycin, Tylosin	Cephapirin, Cloxacillin, Penicillin-G, Demeclocycline, Doxycycline, Methacycline, Rolitetracycline
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	luxuriant	>70%	Gentamycin, Netilmycin, Sisomycin, Paromomycin	Neomycin, Novobiocin

## Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on label.

## Reference

1. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Edi, Tata McGraw-Hill Publishing Company Ltd, New Delhi
2. The United States Pharmacopoeia 2011, USP 34/NF 29, The United States Pharmacopoeial Convention, Rockville, MD.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
5. European Pharmacopoeia, 2011, European Department, for the Quality of Medicines
6. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
7. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242- 259 (April 1).

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