



Technical Data

Antibiotic HiVeg Assay Medium No. 10 (Polymyxin Seed HiVeg Agar) MV225

Antibiotic HiVeg Assay Medium No. 10 (Polymyxin Seed HiVeg Agar) is used as seed layer medium for assaying the products containing Polymyxin B, also for assaying Carbenicillin, Colistin and Colistimethate Sodium.

Composition**

Ingredients	Gms / Litre
HiVeg hydrolysate	17.000
Papaic digest of soyabean meal	3.000
Sodium chloride	5.000
Dextrose	2.500
Dipotassium phosphate	2.500
Agar	12.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42 grams in 1000 ml purified/distilled water containing 10 ml of Polysorbate 80. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Antibiotic HiVeg Assay Medium No. 10 is prepared by incorporating vegetable peptones in place of animal peptones, making the medium BSE-TSE risks free. This can be used for the same purpose of Antibiotic Assay Medium No. 10 for the performance of various antibiotic assays. Grove and Randall have elaborately elucidated the methods to perform these assays and various media used for that (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (2). Antibiotic HiVeg Assay Medium No. 10 is used as a seed agar for assay of Polymyxin B, Colistin methate sodium, Colistin & carbenicillin.

All conditions in the microbiological assay must be carefully controlled. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and 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 Polymyxins are reported to have slow diffusion in agar giving smaller zone of inhibition (3). However, the reduced agar concentration (1.2%) in this medium improves the diffusion of Polymyxin in the medium. Polysorbate 80 is reported to function synergistically with Polymyxins against spheroplasts of *Pseudomonas aeruginosa*. Polysorbate 80 enhances the penetration of Polymyxin through the cytoplasmic membrane and hence is an appropriate ingredient in the medium used for assay of Polymyxin (4).

Nutrients and growth factors are supplied by the ingredients like casein enzymic HiVeg hydrolysate and papaic digest of soyabean meal. Sodium chloride maintains the osmotic equilibrium. Dipotassium phosphate provides the buffering system. Dextrose serves as the source of energy.

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 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.2% w/v aqueous solution containing 1% polysorbate 80 at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Cultural Response <i>Bordetella bronchiseptica</i> ATCC 4617	50-100	luxuriant	>=50%	Polymyxin B, Colistimethate sodium, Colistin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	>=70%	Carbenicillin

Storage and Shelf Life

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

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc, New York.
2. Schmidt and Moyer, 1944; J. Bact, 47:199.
3. Barry, 1991, Antibiotics in Laboratory Medicine, New York pp3.
4. Brown & Wesley 1968, J. Gen. Microbiology, 1968,50(3) Supp:ix.

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