HIMEDIA®

Technical Data

Antibiotic HiVeg™ Assay Medium No. 5 (Streptomycin HiVeg™ Assay Agar w/ Yeast Extract)

MV006

Intended use

Recommended for microbiological assay of Dihydrostreptomycin, Framycetin and Kanamycin B using *Bacillus subtilis*

Composition**

Ingredients	Gms / Litre
HiVeg™ peptone	6.000
HiVeg™ extract	1.500
Yeast extract	3.000
Agar	15.000
Final pH (at 25°C)	7.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.5 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates or dispense as desired.

Advice: Recommended for the Microbiological assay of Dactinomycin, Dihydrostreptomycin, Kanamycin B, Streptomycin, and Framycetin.

Principle And Interpretation

This medium is commonly used for assaying Streptomycin by cylinder plate method using *Bacillus subtilis* as test organism. This method is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds etc. Medium composition is in accordance to the specifications detailed in the FDA (5) and numerically identical to the name assigned by Grove and Randall (1). Antibiotic HiVeg™ Assay Medium No. 5 is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg™ Peptone, yeast extract and HiVeg™ extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other necessary growth nutrients for the test organism like *Bacillus subtilis.* The medium provides solidified substratum for growth of organisms. The pH-7.9 maintained in this medium- provides optimum growth conditions for *Bacillus subtilis* (4). This medium is used to prepare the base as well as seed layer in the microbiological assay of antibiotics such as Dihydrostreptomycin, Framycetin and Kanamycin B. To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized test culture can be overlaid. Even distribution of the layer is important.

Type of specimen

Pharmaceutical preparations

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (2,5,6).
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. Freshly prepared plates must be used or it may result in erroneous results.

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 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 2.55% w/v aqueous solution at 25°C. pH : 7.9±0.2

pH

7.70-8.10

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	good-luxuriant	>=70%	Dihydrostreptomycin, Framycetin, Kanamycin B

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
3. 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.
4. Stearn and Stearn, J Bacteriol. 1933. 26(1): 37-55.
5. 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).
6. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention,Rockville, MD.

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