PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium E (DM751)

Intended Use
Antibiotic Assay Medium E (DM751) is recommended for microbiological assay of Neomycin sulphate and Framycetin sulphate using *Bacillus subtilis* and *Bacillus pumilus*.

Product Summary and Explanation
Antibiotic assay media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[1] The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[2] An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms. Biological tests offer the most convenient means of performing an assay,[3] since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods.[4] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[5] for the assay of penicillin, was later modified by Foster and Woodruff[6] and by Schmidt and Moyer.[7] 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.[8]
Antibiotic Assay Medium E is widely used as seed agar in the plate assay of Framycetin sulphate and Neomycin sulphate using *Bacillus subtilis* and *Bacillus pumilus* as test organism. This medium is formulated in accordance to British Pharmacopoeia and European Pharmacopoeia.[9,10]

Principles of the Procedure
Antibiotic Assay Medium E contains combination of peptic digest of animal tissue and meat extract which provides nitrogenous, amino acids and other essential growth nutrients. Phosphates are incorporated in the medium to provide good buffering action. The low concentration of agar facilitates proper diffusion of antibiotic in the seed agar.
Cylinder Plate Assay
This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. After incubation the diameter of a zone of inhibition depends, in part, on the concentration or activity of the antibiotic. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.
Prediffusion of antibiotics for 10-20 mins in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotics followed by incubation of plates for microbial growth.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue (Peptone)	5.00
Meat extract	3.00
Disodium hydrogen phosphate.12H_2O	26.90
Agar	10.00
Final pH: 7.9 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Freshly prepared plates should be used for antibiotic assays.
4. All conditions in the microbiological assay must be controlled carefully.
5. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions
1. Suspend 28.67 grams in one litre of purified/distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 2.87% solution	pH : 7.9 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

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

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Antibiotic Assayed
1.	*Bacillus pumilus NCTC 8241*	50 – 100	good-luxuriant	>=70%	Neomycin sulphate, Framycetin sulphate
2.	*Bacillus subtilis ATCC 6633*	50 – 100	good-luxuriant	>=70%	Neomycin sulphate, Framycetin sulphate

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Preparation of Stock cultures

1. Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
2. Using sterile purified water, saline or Antibiotic Medium No. 3, prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant and further dilute the culture to obtain the desired organism concentration.
3. In some turbidimetric assays, an 18- 24 hour culture of the test organism grown in Antibiotic Assay Medium No. 3, diluted to obtain the optimal number of organisms, is used.
4. For using *Bacillus subtilis* as a test organism, inoculate the organism on Antibiotic Assay Medium No. 1 and incubate at 35-37°C for 1 week. Wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Using sterile purified water, wash the spores three times, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. Inoculum prepared following this method should produce a sharp zone in the assay.
5. For preparing spore suspension of *B. subtilis*, Antibiotic Assay Medium No. 1 modified by the addition of 300mg manganese sulfate ($MnSO_4 \cdot H_2O$) per liter is used which aids in the sporulation of *B. subtilis*.

Cylinder Plate Assay

1. Use 20 × 100 mm glass or plastic Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover.
2. Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long. Clean the cylinders carefully to remove all residues, using an occasional acid bath (i.e., with approximately 2N nitric acid or with chromic acid).
3. Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.
4. For assuring accurate assays, use a level surface for working to obtain uniformly thick base and seed layers in the Petri dish.
5. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 ml base layer and a 4 ml seed layer.
6. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Results

1. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic.
2. Refer to appropriate references and specific test procedures.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Antibiotic Assay Medium E
Product Code : DM751
Available Pack sizes : 500gm

References

1. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
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6. Foster and Woodruff. 1943. J. Bacteriol. 46:187.
7. Schmidt and Moyer. 1944. J. Bacteriol. 47:199.
8. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
9. British Pharmacopoeia, 2011, The Staionery Office , British Pharmacopoeia.
10. European Pharmacopoeia, 2011, European Department, for the Quality of Medicines.

Further Information

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DM751PSS,QAD/FR/024,Rev.00

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