PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No.36 (DM072)

Intended Use
Antibiotic Assay Medium No.36 (DM072) is used for isolating a wide variety of fastidious organisms, when used with or without blood or other enrichments.

Product Summary and Explanation
Antibiotic assay media are prepared according to the specifications of the USP[1], European Pharmacopeia[2] and AOAC International.[3] The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[4] The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[1] 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,[5] since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods.[6] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[7] for the assay of penicillin, was later modified by Foster and Woodruff[8] and by Schmidt and Moyer.[9] 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.[10]
The composition of this medium is in accordance to CFR.[11] This medium is recommended for sterility testing.[12] Antibiotic Assay Medium No. 36 is widely employed as seed agar for agar diffusion assay for antibiotic bleomycin. *Mycobacterium smegmatis* the test organism is also maintained in this medium. This medium is employed for cultivation and isolation of fastidious or non-fastidious microorganisms and also used as maintenance medium of *Psuedomonas aeruginosa* for plate assay of ticarcillin. Like the conventional medium, Antibiotic Assay Medium No. 36 is used for a multitude of purposes including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and a base for media containing blood.[13,14]

Principles of the Procedure
Antibiotic Assay Medium No. 36 contains casein enzymic hydrolysate and papaic digest of soyabean meal which provides nitrogenous growth factors, vitamins and other essential growth nutrients. Sodium chloride helps to maintain the osmotic balance of the medium. Agar provides excellent solid substratum for support and overlayering of seed agar, for the assay of Bleomycin. Addition of glycerol is important for provision of carbon to the test organism.
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
Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Sodium chloride	5.00
Agar	15.00
Final pH: 7.3 ± 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 40 grams of the medium in one liter of purified/distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. If desired aseptically add 5% v/v defibrinated blood in previously cooled medium at 45 - 50°C.
5. Mix well before pouring into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal Medium : Light yellow coloured clear to slightly opalescent gel After addition of 5-7%w/v sterile defibrinated blood :Cherry red coloured opaque gel forms in Petri plates
Reaction of 4.0% solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added 5-7%w/v sterile defibrinated blood after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Growth w/ Blood	Recovery w/ Blood
	Growth at 30-35°C for <=3 days					
1.	*Bacillus subtilis ATCC 6633*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
2.	*Staphylococcus aureus ATCC25923*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
3.	*Staphylococcus aureus ATCC 6538*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
4.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
5.	*Escherichia coli ATCC 8739*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
6.	*Escherichia coli NCTC 9002*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
7.	*Pseudomonas aeruginosa ATCC 27853*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
8.	*Pseudomonas aeruginosa ATCC 9027*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
9.	*Salmonella Abony NCTC 6017*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
10.	*Micrococcus luteus ATCC 9341*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
11.	*Salmonella Typhimurium ATCC 14028*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
12.	*Streptococcus pneumonia ATCC 6305*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
	Growth at 20-25°C for <=5 days					
13.	*Aspergillus brasiliensis ATCC 16404*	50-100	good-luxuriant		good-luxuriant	
14.	*Candida albicans ATCC 2091*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%
15.	*Candida albicans ATCC 10231*	50-100	good-luxuriant	>=70%	good-luxuriant	>=70%

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

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. For using as a test organism *Bacillus subtilis*, inoculate the organism on Antibiotic Medium No. 1 and incubate at 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.
4. For preparing spore suspension of *B. subtilis*, Antibiotic Medium No. 1 modified by the addition of 300 mg 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. Antibiotic assay medium No.1 is used as the seed agar.
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 No.36
Product Code : DM072
Available Pack sizes : 100gm/500gm

References

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11. 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, (April1).
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Further Information

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