PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No.37 (DM073U)

Intended Use

Antibiotic Assay Medium No.37 (DM073U) is recommended for cultivation of a wide variety of microorganisms and for sterility testing of mould in compliance with USP.

Product Summary and Explanation

Antibiotic assay media are prepared according to the specifications of the USP[1], European Pharmacopeia[2] and AOAC International[3] and by the FDA.[4] The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.[5] The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[1] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[1] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay was reported by Schmidt and Moyer.[6] Ripperre et al reported that turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than agar diffusion procedures.[7] Antibiotic assay Medium No. 37 is formulated in accordance with The United States Pharmacopoeia[8] and is recommended for the cultivation of a wide variety of microorganisms and sterility testing of pharmaceutical preparations. This medium is also used for the sensitivity testing by the tube dilution method for antimicrobial agents.

Principles of the Procedure

Antibiotic Assay Medium No.37 contain pancreatic digest of casein and soyabean peptone which provides amino acids, long chain peptides and other growth factors making the medium nutritious. Dextrose stimulates the growth by providing carbon and energy. Sodium chloride maintains the osmotic equilibrium of the medium and retains the cell viability and cell integrity. Phosphates in the medium enhance the buffering action.

Turbidimetric Assay

The turbidimetric method is based on the change or inhibition of growth of a microbial culture in a liquid medium containing a uniform solution of an antibiotic. Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. After incubation of the test organism in the working dilutions of the antibiotics, the amount of growth is determined by measuring the light transmittance using spectrophotometer. The concentration of antibiotic is determined by comparing amounts of growth obtained with that given by the reference standard solutions. Use of this method is appropriate only when test samples are clear.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of casein	17.00
Soyabean peptone	3.00
Dextrose	2.50
Sodium chloride	5.00
Dipotassium phosphate	2.50
Final pH: 7.3 ± 0.1 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

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

Directions

1. Suspend 31.5 grams of medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Distribute 10 ml amounts into tubes containing inverted Durham's tubes.
4. Autoclave at 121°C, 15 psi pressure, for 10 minutes.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear solution without any precipitate
Reaction of 3.0% Solution	pH : 7.3 ± 0.1 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed (i) for Bacteria at 30-35°C after 18-48 hours (ii) for Fungi at 20-25°C after 2-5 days

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Staphylococcus aureus ATCC 6538*	50 -100	good-luxuriant
2.	*Escherichia coli ATCC 8739*	50 -100	good-luxuriant
3.	*Pseudomonas aeruginosa ATCC 9027*	50 -100	luxuriant (when incubated anaerobically)
4.	*Bacillus subtilis ATCC 6633*	50 -100	good-luxuriant
5.	*Streptococcus pyogenes ATCC 19615*	50 -100	good-luxuriant
6.	*Candida albicans ATCC 10231*	50 -100	good-luxuriant
7.	*Candida albicans ATCC 2091*	50 -100	good-luxuriant
8.	*Aspergillus brasiliensis ATCC 16404*	50 -100	good-luxuriant

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- 24hour culture of the test organism grown in Antibiotic 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*.

Turbidimetric Assay

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1. Use glass or plastic test tubes (i.e., 16 × 125 mm or 18 × 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.
2. Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.
3. Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used. Prepare working dilutions of the antibiotic reference standards in specific concentrations.
4. To a 1 ml quantity of each solution in a suitable tube, add 9 ml of inoculated broth, as required.
5. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes.
6. Incubate the tubes for 3-4 hours at the required temperature, generally in a water bath. At the end of the incubation period, stop growth by adding 0.5 ml of 1:3 formalin.
7. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer.
8. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.
9. Refer to appropriate procedures outlined in the references for a complete discussion of antibiotic assay methods.

Results

Refer to appropriate references and test procedures for interpretation of results.

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.37
Product Code : DM073U
Available Pack sizes : 100gm / 500gm

References

1. 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.
2. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourgh, France.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. 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 pg 242-259 (April 1).
5. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
6. Schmidt and Moyer, 1944; J. Bact, 47:199.
7. Rippere R. A.. Some principles of microbiological turbidimetric assays of antibiotics. J. Assoc. off. Anal. Chem. 1979. 62(4):951-6.

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8. United States Pharmacopoeia 1985, US Pharmacopoeial Convention, Inc.,Rockville, MD.
9. Wright and Welch, 1959-60, Antibiotics Ann., 61.

Further Information

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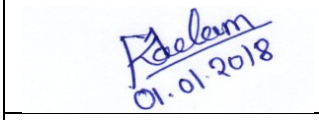	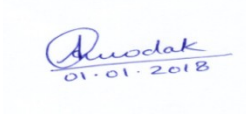	
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