PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No.4 (Yeast Beef Agar) (DM017)

Intended Use

Antibiotic Assay Medium No. 4 (Yeast Beef Agar) (DM017) is recommended for detection of Penicillin-G in milk sample using *Bacillus stearothermophilus*.

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] 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 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] This dehydrated culture medium is suitable for plate counts in pharmaceutical and related products and for the microbial assay and detection of antibiotics like penicillin in milk. Antibiotic Assay Medium no. 4 is used in a cylinder plate method for detecting penicillin in nonfat dry milk.[8] To achieve satisfactory test results, the use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics.

Principles of the Procedure

Antibiotic Assay Medium no. 4 contains peptone, yeast and beef extract which provides nutritional requirement for growth of the indicator organism like *Bacillus stearothermophilus*, *Micrococcus luteus*. This medium is similar to Antibiotic assay medium no. 2 except for the additional ingredient dextrose which serves as an easily available source of carbon that stimulates luxuriant growth of the test organisms.
Generally, presence of penicillin in milk is detected by the cylinder plate method, using *Micrococcus luteus* as the test organism, and by paper disk method, using *Bacillus stearothermophilus*. The cylinder plate method is recommended as the standard for quantification of ß-lactam residues. A description of the cylinder plate method for detecting penicillin in dry powdered milk is given by Kramer et al.[9] The same basic procedure is also recommended to the assay of penicillin in fluid milk. The use of this medium assures well defined zones of 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 is observed which 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.
Pre-diffusion of antibiotics for 10-20 minutes 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
Peptone	6.00
Yeast extract	3.00
Beef extract	1.50
Dextrose	1.00
Agar	15.00
Final pH: 6.6 ± 0.1 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 26.50 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 2.65% solution	pH 6.6 ± 0.1 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Growth Promotion is carried out in accordance with USP. Cultural characteristics observed after an incubation at 32-35°C for 18-24 hours. Recovery rate is considered as 100% for bacterial growth on Soyabean Casein Digest Agar and fungal growth on Sabouraud Dextrose Agar.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Incubation Temperature	Incubation Period
1.	*Micrococcus luteus ATCC 10240*	50-100	good-luxuriant	>=50%	32-35°C	18-24 hours
2.	*Bacillus stearothermophilus ATCC 7953*	50-100	good-luxuriant	>=50%	55°C	18-24 hours

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 Assay Medium 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 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 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 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*.
6. When *B. cereus* var. *mycoides* is required, inoculate the organism on Antibiotic Assay Medium 1 and incubate at 30°C for 1 week. Wash and prepare the spores as for *B. subtilis*, above.

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
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
For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.[1,2,3]

Packaging
Product Name : Antibiotic Assay Medium No.4 (Yeast Beef Agar)
Product Code : DM017
Available Pack sizes : 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. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett *ii*:177.
6. Foster and Woodruff. 1943. J. Bacteriol. 46:187.
7. Schmidt and Moyer. 1944. J. Bacteriol. 47:199.
8. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C
9. Kramer, J., G.G. Carter, B. Arret, J. Wilner, W.W. Wright, and A. Kirshbaum. 1968. Antibiotic residues in milk, dairy products and animal tissues: methods, reports and protocols. Food and Drug Administration, Washington, DC.

Further Information
For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.