

PRODUCT SPECIFICATION SHEET

Antibiotic Assay Medium No. 40 (DM211U)

Intended Use

Antibiotic assay medium No. 40 (DM211U) is used in microbiological assay of Thiostrepton using *Enterococcus hirae* as test organism in accordance with United States Pharmacopoeia.

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 No. 40 is used as maintenance medium for test organism *Enterococcus hirae* ATCC 10541 used for assay of Thiostrepton. It is prepared in accordance with USP.⁽⁴⁾

Principles of the Procedure

Antibiotic Assay Medium No. 40 contains combination of peptic digest of animal tissue, casein enzymic hydrolysate, and yeast extract which provides nitrogenous growth factors, vitamins and other essential growth nutrients. Dextrose serves as energy and carbon source for enhancing the growth of test organism. Phosphates in the medium provide good buffering action required during maintenance of the test organisms. Incorporation of polysorbates reduces the surface tension, maintaining uniform suspension of cells and also can neutralize phenolic compounds in the test sample, if any.

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.

Pre-diffusion 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
Yeast extract	20.00
Polypeptone	5.00
Dextrose	10.00
Monobasic potassium phosphate	2.00
Polysorbate 80	0.10
Agar	10.00
Final pH: 6.7 ± 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 47.1 grams in one litre of distilled water.
2. Heat to boiling with frequent agitation to dissolve the medium completely.

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- Dispense and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
- Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Light amber coloured slightly opalescent gel forms in Petri plates
Reaction of 4.71% Solution	pH: 6.7 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Growth Promotion Test

As per United States Pharmacopoeia

Expected Cultural Response: Cultural characteristics observed after an incubation at 36-37.5°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Antibiotic Assayed
1.	<i>Enterococcus hirae</i> ATCC 10541	50 - 100	good-luxuriant	≥70%	Thiostrepton

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

Test Procedure

Preparation of Stock cultures

- Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
- 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.

Cylinder Plate Assay

- 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.
- 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).
- Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.
- For assuring accurate assays, use a level surface for working to obtain uniformly thick base and seed layers in the Petri dish.
- 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 2.1 mL base layer and a 4 mL seed layer.
- 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

- After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic.
- 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,

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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.40

Product Code : DM211U

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.
3. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed, Tata McGraw-Hill Publishing Company Ltd, New Delhi.
4. United States Pharmacopoeia 2009, US Pharmacopoeial Convention, Inc., Rockville, MD.
5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancet ii:177.
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., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

Further Information

For further information please contact your local MICROMASTER Representative.



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