Antibiotic Assay Medium G (DM025I)

Intended Use
Antibiotic Assay Medium G (DM025I) is recommended for microbiological assay of Amphotericin B and Nystatin using Saccharomyces cerevisiae as the test organisms in compliance with IP.

Product Summary and Explanation
Antibiotic assay media are prepared according to the specifications of the USP, European Pharmacopeia and AOAC International. The antibiotic media are identified numerically with names assigned by Grove and Randall in Assay Methods of Antibiotics. The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al. for the assay of penicillin, was later modified by Foster and Woodruff and by Schmidt and Moyer. The medium composition of Antibiotic Assay Medium G is in accordance to IP and CFR. This medium is used as seed agar for assay of antifungal agents like Amphotericin B and Nystatin. This medium is used for maintenance and inoculum development of Saccharomyces cerevisiae, indicator organism. This medium is also used for assaying mycostatic activity in pharmaceutical formulations. This medium is formulated as reported by Kirshbam and Arret.

Principles of the Procedure
Antibiotic Assay Medium G contains peptone, yeast and beef extract which provides necessary growth nutrients for the test organisms. Dextrose in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is maintained by sodium chloride which retains the cell integrity and viability. The medium provides solidified substratum for growth of organisms.

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. The results depend on critical rates of diffusion of the antibiotic, critical growth rates of the standard organisms and critical minimal inhibitory coefficient levels of each organism. 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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>9.40</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>4.70</td>
</tr>
<tr>
<td>Beef extract</td>
<td>2.40</td>
</tr>
<tr>
<td>Dextrose</td>
<td>10.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.00</td>
</tr>
<tr>
<td>Agar</td>
<td>23.50</td>
</tr>
</tbody>
</table>

Final pH: 6.1 ± 0.1 at 25°C

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 60 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

<table>
<thead>
<tr>
<th>Dehydrated Appearance</th>
<th>Cream to yellow homogeneous free flowing powder</th>
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<tbody>
<tr>
<td>Prepared Medium</td>
<td>Yellow coloured clear to slightly opalescent gel forms in Petri plates</td>
</tr>
<tr>
<td>Reaction of 6.0% solution</td>
<td>pH 6.1 ± 0.1 at 25°C</td>
</tr>
<tr>
<td>Gel Strength</td>
<td>Firm, comparable with 2.35% Agar gel</td>
</tr>
</tbody>
</table>

Expected Cultural Response: Cultural characteristics observed after an incubation at 29-31°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Organisms</th>
<th>Results to be achieved</th>
<th>Antibiotics Assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saccharomyces cerevisiae ATCC 2601</td>
<td>50-100</td>
<td>good-luxuriant</td>
</tr>
<tr>
<td>2.</td>
<td>Saccharomyces cerevisiae ATCC 9763</td>
<td>50-100</td>
<td>good-luxuriant</td>
</tr>
</tbody>
</table>

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.

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 standard test procedures for interpretation of results.

Storage
Store the sealed bottle containing the dehydrated medium at 2 - 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 G
Product Code: DM025I
Available Pack sizes: 500gm

References
5. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett 111: 177.

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.
Email: micromaster@micromasterlab.com

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