Antibiotic Assay Medium No.5 (Streptomycin Assay Agar w/ Yeast Extract)

Antibiotic Assay Medium No.5 (Streptomycin Assay Agar w/Yeast extract) is used for microbiological assay of Dihydrostreptomycin, Framycetin and Kanamycin B using Bacillus subtilis

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>6.000</td>
</tr>
<tr>
<td>(Peptone)</td>
<td></td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.9±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.50 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Advice: Recommended for the Microbiological assay of Dihydrostreptomycin, Framycetin, Kanamycin

Principle And Interpretation

This medium is commonly used for assaying Streptomycin by cylinder plate method using Bacillus subtilis as test organism. This method is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds etc. Medium composition is in accordance to the specifications detailed in the FDA (1) and numerically identical to the name assigned by Grove and Randall (2).

Peptic digest of animal tissue, yeast and beef extract provides necessary growth nutrients for the test organisms like Bacillus subtilis.

The medium provides solidified substratum for growth of organisms. The pH-7.9 maintained in this medium provides optimum growth conditions for Bacillus subtilis. This medium is used to prepare the base as well as seed layer in the microbiological assay of antibiotics such as Dihydrostreptomycin, Framycetin and Kanamycin B.

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized test culture can be overlaid. Even distribution of the layer is important.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 2.55% w/v aqueous solution at 25°C. pH : 7.9±0.2

pH
7.70-8.10

Cultural Response
M006: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Antibiotics assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultural Response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633 50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>Dihydrostreptomycin, Framycetin, Kanamycin B</td>
<td></td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

**Reference**