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 (Peptone)</td>
<td>6.000</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><strong>Final pH (at 25°C)</strong></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* (3). 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.
### Organism

<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><em>Bacillus subtilis ATCC 6633</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>Dihydrostreptomycin, Framycetin, Kanamycin B</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


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**Disclaimer:**

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