Antibiotic Assay Medium E

Antibiotic Assay Medium E is used for microbiological assay of Framycetin and Kanamycin B using *Bacillus subtilis* in accordance with Indian Pharmacopoeia

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>6.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>pH after sterilization</td>
<td>7.9±0.1</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

**Directions**

Suspend 25.5 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 Framycetin and Kanamycin B.

**Principle And Interpretation**

This medium 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 IP, FDA (1,2). Peptone, yeast and beef extract provide necessary growth nutrients for the test organisms like *Bacillus subtilis*. This 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 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 coloured homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Medium amber coloured slightly opalescent gel forms in Petri plates.

**pH**

7.80-8.00

**Cultural Response**

MM006: Cultural characteristics observed after an incubation at 32-35°C for 5 days.

<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</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>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

1. Indian Pharmacopeia 2010, Ministry of Health and Family welfare, Government of India, New Delhi.