Antibiotic Assay Medium E is used in the microbiological assay of Neomycin sulphate and Framycetin sulphate using *Bacillus subtilis* and *Bacillus pumilus*.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue (Peptone)</td>
<td>5.000</td>
</tr>
<tr>
<td>Meat extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate.12H2O</td>
<td>26.900</td>
</tr>
<tr>
<td>Agar</td>
<td>10.000</td>
</tr>
<tr>
<td><strong>Formula adjusted, standardized to suit performance parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.9±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 28.67 grams of dehydrated medium 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.

**Principle And Interpretation**

Antibiotic Assay Medium E is widely used as seed agar in the plate assay of Framycetin sulphate and Neomycin sulphate using *Bacillus subtilis* and *Bacillus pumilus* as test organism.

Peptic digest of animal tissue (Peptone) and meat extract supplies nutrients essential for microbial growth. Phosphates are incorporated in the medium to provide good buffering action. The low concentration of agar facilitates proper diffusion of antibiotic in the seed agar. This medium is formulated in accordance to British Pharmacopoeia (1) and European Pharmacopoeia (2).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. Zones of inhibition around the antibiotic are then measured. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results.

**Quality Control**

**Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.0% Agar gel.

**Colour and Clarity of prepared medium**

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 2.87 % w/v aqueous solution after sterilization. pH : 7.9±0.2

**pH**

7.70 - 8.10

**Cultural Response**

M1347: 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><em>Bacillus pumilus NCTC 824150-100</em></td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td></td>
<td>Neomycin sulphate,</td>
</tr>
</tbody>
</table>
Framycetin sulphate
Neomycin sulphate,
Framycetin sulphate

Bacillus subtilis ATCC 6633  50-100 luxuriant  >=70%

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
2. European Pharmacopoeia, 2009, European Department, for the Quality of Medicines.

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