Antibiotic Assay Medium No. 32

Antibiotic Assay Medium No. 32 is recommended for preparing inoculum of *Bacillus subtilis* to be used as test organism for assaying Dihydrostreptomycin and Vancomycin by plate assay method in accordance with United States 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>Pancreatic digest of casein</td>
<td>4.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.000</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 6.6±0.1

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

**Directions**

Suspend 30.8 gms in 1000ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilise by autoclaving at 15lbs pressure (121°C) for 15 minutes.

*Advice: Recommended for the microbiological assay of Dihydrostreptomycin and Vancomycin.*

**Principle And Interpretation**

This medium is formulated in accordance to USP and FDA (1,2) and is a modification of Antibiotic assay medium No.1. This medium is used to develop inoculum of *Bacillus subtilis* for antibiotic assay.

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by peptone, pancreatic digest of casein, yeast and beef extract. Dextrose in the medium serves as the carbon source for stimulating the growth of the test microorganism. Manganese sulphate in this medium facilitates the sporulation and growth of *Bacillus subtilis* (3,4,5), which is generally used as test organisms for plate assay of Dihydrostreptomycin and Vancomycin.

**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**

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

**Reaction**

Reaction of 3.08% w/v aqueous solution. pH: 6.6±0.1

**pH**

6.50-6.70

**Cultural Response**

MU1141: 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> ATCC 6633</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>Dihydrostreptomycin, Vancomycin</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