Antibiotic Medium No. 2

Antibiotic Medium No. 2 is used as basal medium for microbiological assay of antibiotics 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>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 (at 25°C)</td>
<td>6.6±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.

**Principle And Interpretation**

This medium is commonly used as base agar for microbiological agar diffusion assays for wide variety of antibiotics. Agar diffusion assays can be performed by cylinders, punched-hole or paper disc tests. This medium is identical numerically with the name assigned by Grove and Randall (1). This medium is prepared according to the specifications detailed in the USP and CFR (2,3).

Peptone, yeast and beef extract nitrogenous, vitamins and mineral requirement for the growth of test organisms. This medium provides solidified substratum for growth of organisms and supports the overlayering of soft agar.

To perform an antibiotic assay the Antibiotic assay medium No. 2 is used as Base Agar. This medium 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, Antibiotic assay medium No.1 as seed agar, inoculated with the standardized 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**

Amber coloured slightly opalescent gel forms in Petri plates.

**pH**

6.50-6.70

**Cultural Response**

MU005: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Basal layer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micrococcus luteus</em> ATCC 10240</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>Bacitracin</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 9144</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>Tylosin</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Staphylococcus aureus**
*ATCC 29737*

50-100 luxuriant >=70%

Amikacin, Cephalothin, Cephapirin, Cloxacillin, Cycloserine, Chlorotetracycline, Demeclocycline, Doxycycline, Kanamycin, Methacycline, Nafcillin, Oxytetracycline, Rolitetracycline, Tetracycline

**Staphylococcus epidermidis**
*ATCC 12228*

50-100 good-luxuriant >=70%

Novobiocin

**Klebsiella pneumoniae**
*ATCC 10031*

50-100 luxuriant >=70%

Capreomycin, Streptomycin, Troleandomycin

**Enterococcus hirae**
*ATCC 10541*

50-100 luxuriant >=70%

Gramicidin, Thio strepton, Tobramycin

**Escherichia coli**
*ATCC 10536*

50-100 luxuriant >=70%

Chloramphenicol, Spectinomycin

---

**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**


---

**Disclaimer:**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.