Phenylethyl Blood Agar Base (Anaerobic) is used for cultivation of fastidious anaerobic bacteria.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>15,000</td>
</tr>
<tr>
<td>Papaic digest of soyabean meal</td>
<td>5,000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5,000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5,000</td>
</tr>
<tr>
<td>Phenylethyl alcohol</td>
<td>2,500</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0,400</td>
</tr>
<tr>
<td>Vitamin K1</td>
<td>0,010</td>
</tr>
<tr>
<td>Hemin</td>
<td>0,005</td>
</tr>
<tr>
<td>Agar</td>
<td>20,000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.5±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 52.92 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C. Aseptically add sterile 50 ml defibrinated sheep or rabbit blood. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Phenylethyl alcohol is a chemical agent that exhibits inhibitory action against gram-negative and certain gram-positive bacteria. Phenylethyl Blood Agar Base (Anaerobic) is used for the isolation of obligate anaerobic gram-positive and gram-negative bacteria (2). Supplementation of medium with L-cystine permits growth of certain thiol-dependent or sulphur containing amino acids- requiring bacteria (2, 3) and fastidious Streptococci. This medium inhibits facultative anaerobic gram-negative bacteria such as *E. coli* and *Proteus* species.

Casein enzymic hydrolysate and papaic digest of soyabean meal provide nitrogen, carbon, sulfur and trace elements to the growing organisms. Addition of sheep blood provides many growth factors. Sodium chloride maintains osmotic equilibrium. Addition of phenylethanol to a nutritive medium permits the growth of gram-positive organisms but inhibits the gram-negative organisms found in the same specimen (1). Phenylethyl alcohol exerts inhibitory bacteriostatic action on gram-negative bacteria by inhibiting their DNA synthesis (4). Addition of hemin, vitamin K1 and L-cystine makes the medium more nutritious and suitable for the growth of fastidious anaerobic bacteria.

**Quality Control**

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 2.0% Agar gel

**Colour and Clarity of prepared medium**
Basal medium: Light amber coloured clear to slightly opalescent gel. After addition of 5% w/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates

**Reaction**
Reaction of 5.29% w/v aqueous solution at 25°C. pH : 7.5±0.2

**pH**
7.30-7.70

**Cultural Response**
M540: Cultural characteristics observed in an anaerobic condition with added 5% w/v sterile defibrinated blood after an incubation at 35-37°C for 48-72 hours (longer if necessary).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td><em>Clostridium butyricum</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>50-100</td>
<td>fair-good</td>
<td>30-40%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store below 8°C and the prepared medium at 2-8°C. Use before expiry date on the label.

**Reference**

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