Enterococcus Presumptive Broth

Enterococcus Presumptive Broth is recommended for detecting the presence of Enterococci in water supplies and other materials of sanitary importance.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.400</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.032</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>8.4±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 15.43 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Warning:** Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

**Principle And Interpretation**

Enterococcus Presumptive Broth is formulated by Sandholzer and Winter (1) for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococci are differentiated from other Streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C (2).

Casein enzymic hydrolysate, yeast extract, dextrose provide essential growth nutrients for Enterococci. Sodium azide inhibits gram-negative organisms. The positive presumptive tests are confirmed by inoculating from Enterococcus Presumptive Broth to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory Agar, M392) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, M394). A negative catalase test is considered confirmed positive evidence of the presence of Enterococci. Single strength medium can be used for small inoculum. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci which is confirmed by inoculating in/on Confirmatory Broth/Agar (M394, M392).

**Quality Control**

**Appearance**
Greenish yellow to light blue homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Blue coloured, clear solution without any precipitate

**Reaction**
Reaction of 1.54% w/v aqueous solution at 25°C. pH : 8.4±0.2

**pH**
8.20-8.60

**Cultural Response**

M419: Cultural characteristics observed after an incubation at 45°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
</tbody>
</table>
Enterococcus faecalis ATCC 50-100
29212

good-luxuriant positive
reaction, yellow
colour

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a

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