EE Broth, Mossel

**Intended Use:**
Recommended for selective enrichment of *Enterobacteriaceae* in the bacteriological examination of food.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>6.450</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.000</td>
</tr>
<tr>
<td>Bile, purified#</td>
<td>20.000</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0135</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

# Equivalent to Ox bile, purified

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

**Directions**
Suspend 43.46 grams in 1000 ml purified / distilled water. Dispense in tubes or flasks as desired. Stopper with cotton plugs or loose fitting caps. Heat in free flowing steam or boiling water for 30 minutes. Cool to 45-50°C. Avoid overheating of the medium. DO NOT AUTOCLAVE.

**Principle And Interpretation**

The family *Enterobacteriaceae* consists of *Salmonella*, *Shigella* and other enteric pathogens. These organisms find entry into the food system through faecally contaminated water. Majority of these organisms may be eliminated under the stringent food processing parameters. But some of these organisms may become sublethally injured during the changes in pH, exposure to steam or heat and other unfavourable conditions (6). Therefore the important aspect of food monitoring depends upon the identification and enumeration of these injured cells, after resuscitation. EE Broth, Mossel, formulated by Mossel et al. (4) is recommended as an enrichment medium for *Enterobacteriaceae* in the biological examination of foods (4) and animal feed stuffs (10).

Peptone and dextrose provide the essential nutrients required for the growth of most of the members of *Enterobacteriaceae*. Brilliant green and Bile, purified, purified inhibit growth of gram-positive bacteria. Lactose-negative, anaerogenic lactose-positive or late lactose-fermenting *Enterobacteriaceae* are often missed by the standard coli-aerogenes test. To overcome this problem, lactose is replaced by dextrose in these media. Phosphates form the buffering system of the medium. The cells damaged while drying or low pH are resuscitated in well-aerated Tryptone Soya Broth (M011) for 2 hours at 25°C prior to enrichment in EE Broth. The resuscitation procedure is recommended for dried foods (5), animal feeds (7) and semi-preserved foods (8). EE Broth is an enrichment broth and should be used in conjunction with Violet Red Bile Glucose Agar (M581).

Subcultures must be made onto lactose differential media such as MacConkey Agar (M081), Deoxycholate Citrate Agar (M065) or Brilliant Green Agar (M016) for the detection of lactose negative or delayed lactose fermenters. This is used to inoculate MPN tubes prepared using EE Broth. Inoculate a loopful from these tubes onto Violet Red Bile Glucose Agar (M581) after an initial incubation at 35-37°C for 24 hours. Typical pink colonies from M581 are subcultured for biochemical confirmation by oxidase and fermentation reactions (1). Decimal dilutions of the food homogenate are used if the expected counts are high or else initial suspension is used. EE Broth, Mossel (M287).

**Type of specimen**

Food samples
Specimen Collection and Handling
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Avoid overheating of the medium as media is heat sensitive.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Emerald green coloured, clear solution without any precipitate

pH
7.00-7.40

Cultural Response
Cultural response was observed after an incubation at 35-37°C for 24-48 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 ATCC 8739</em> (00012*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>positive reaction, yellow colour</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 9027</em> (00026*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus ATCC 6538 (00032</em>)*</td>
<td>$\geq 10^4$</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em> (00013*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>positive reaction, yellow colour</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC NCTC 9002</em></td>
<td>50 -100</td>
<td>luxuriant</td>
<td>positive reaction, yellow colour</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 27853</em> (00025*)*</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella aerogenes ATCC 13048</em> (00175*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>positive reaction, yellow colour</td>
</tr>
<tr>
<td><em>Proteus mirabilis ATCC 25933</em></td>
<td>50 -100</td>
<td>luxuriant</td>
<td>positive reaction, yellow colour</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 15 - 25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

**Reference**


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**Disclaimer**

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