Purple Agar Base

Intended Use:
Recommended for preparation of carbohydrate media used in fermentation studies for the cultural identification of pure cultures of enteric and other microorganisms.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone special</td>
<td>10.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Bromo cresol purple</td>
<td>0.020</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

\# - Equivalent to Meat extract B

Directions
Suspend 31.02 grams in 1000 ml purified / distilled water. Add 5 - 10 grams of the carbohydrate to be tested. Heat to boiling to dissolve the medium completely. Dispense in tubes as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Alternatively sterilize the basal medium prepared using 900 ml purified / distilled water and add 100 ml separately sterilized 5 - 10% solution of the desired carbohydrate to it.

Principle And Interpretation
Purple Agar Base is used for studying carbohydrate fermentation reactions, particularly in the identification of gram-negative enteric bacteria on addition of the desired carbohydrate (1, 4). Purple media were originally formulated by Vera (8) and further modified by addition of HM peptone B (3). These media are recommended by FDA (2) for fermentation studies of sugars.

HM peptone B and peptone special supply nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients especially nitrogen sources to the growing organisms. Sodium chloride maintains the osmotic balance of the medium. Bromocresol purple is the pH indicator, which turns yellow at acidic pH. Gas production is evident by splitting of agar. The acid produced during the fermentation of carbohydrate causes bromocresol purple, the pH indicator to turn yellow. If the carbohydrate is not utilized or fermented, the color of the medium remains unchanged or becomes more alkaline (darker purple) due to decarboxylation of the amino acids present in the medium. It is recommended (7) to add carbohydrate in 1% concentration to avoid possible reversion reactions except glucose (dextrose). If the medium containing carbohydrate is sterilized by autoclaving, precautions should be taken to use minimum amount of heat required for sterilization to avoid hydrolysis of the carbohydrate.

Type of specimen
Isolated Microorganisms

Specimen Collection and Handling:
For isolated Microorganisms samples follow appropriate techniques for handling specimens as per established guidelines (5,6). 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. It is recommended (6) to add carbohydrate in 1% concentration to avoid possible reversion reactions except glucose (dextrose).
2. If the medium containing carbohydrate is sterilized by autoclaving, precautions should be taken to use minimum amount of heat required for sterilization to avoid hydrolysis of the carbohydrate.

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
Cream to greenish yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent gel forms in tubes as slants
Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.8±0.2
pH
6.60-7.00

Cultural Response
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>Acid (without carbohydrate)</th>
<th>Gas (without carbohydrate)</th>
<th>Acid (with 1% dextrose)</th>
<th>Gas (with 1% dextrose)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction</td>
<td>positive reaction, yellow colour</td>
<td>positive reaction</td>
</tr>
<tr>
<td>ATCC 19112</td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction</td>
<td>positive reaction, yellow colour</td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Neisseria meningitidis ATCC 13090</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction</td>
<td>positive reaction, yellow colour</td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus ATCC 25923</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative reaction, no colour change</td>
<td>negative reaction</td>
<td>positive reaction, yellow colour</td>
<td>negative reaction</td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. 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 (5,6).

Please refer disclaimer Overleaf.
Reference