Phenol Red HiVeg™ Agar Base

**Intended Use:**
Recommended as a basal medium to which carbohydrate can be added for fermentation studies of microorganisms.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ peptone No. 3</td>
<td>10.000</td>
</tr>
<tr>
<td>HiVeg™ extract</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 31.02 grams in 1000 ml purified / distilled water. Add 5-10 grams of carbohydrate as desired. Heat to boiling to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed media to cool in slanted position to form slants with deep butts.

**Note:** For critical studies, it is recommended to use filter sterilized carbohydrate which is to be incorporated aseptically in sterile medium base.

**Principle And Interpretation**
Phenol Red Agar media are recommended (1,2,5) for studying the fermentation of various carbohydrates individually by the pure cultures of microorganisms. Phenol Red HiVeg™ Agar Base is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associated with animal peptones.

HiVeg™ peptone No. 3 and HiVeg™ extract which is free from fermentable carbohydrates is added in the medium thereby preventing the production of false positive reactions. Phenol Red HiVeg™ Agar when supplemented with a specific carbohydrate, a positive carbohydrate fermentation reaction is indicated by the production of a yellow colour in agar due to the effect of acid production. Gas production is indicated by the splitting of agar or by the bubbles formation. Plates or tubes may be incubated aerobically or anaerobically depending on the type of the test organism. Addition of some carbohydrates may result in an acid reaction and hence 0.1N sodium hydroxide can be added dropwise to restore the original colour taking care not to obtain too deep red or cerise colour.

**Type of specimen**
Isolated Microorganism

**Specimen Collection and Handling:**
For isolated microorganism samples follow appropriate techniques for handling specimens as per established guidelines (3,4). 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. Addition of some carbohydrates may result in an acid reaction and hence 0.1N sodium hydroxide can be added dropwise to restore the original colour taking care not to obtain too deep red or cerise colour.
## 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 pink homogeneous free flowing powder

### Gelling
firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in tubes as slants

### Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

### pH
7.20-7.60

### Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>without carbohydrate, (Acid)</th>
<th>without carbohydrate, (Gas)</th>
<th>with dextrose, (Acid)</th>
<th>with dextrose, (Gas)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alcaligenes faecalis ATCC 8750</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Negative reaction</td>
<td>Negative reaction</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922 (00013</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Positive reaction, yellow reaction</td>
<td>Positive reaction</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae ATCC 13883 (00097</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Positive reaction, yellow reaction</td>
<td>Positive reaction</td>
</tr>
<tr>
<td><em>Proteus vulgaris ATCC 13315</em></td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Positive reaction, yellow reaction</td>
<td>Positive reaction</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC 14028 (00031</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Positive reaction, yellow reaction</td>
<td>Positive reaction</td>
</tr>
<tr>
<td><em>Shigella flexneri ATCC 12022 (00126</em>)*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Negative reaction, no colour change</td>
<td>Negative reaction</td>
<td>Positive reaction, yellow reaction</td>
<td>Positive 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 20-30°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 (3,4).
Reference


Revision : 01 / 2019

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