### Intended Use

Phenol Red Agar Base is used with added carbohydrate in differentiating pure cultures of bacteria based on fermentation reactions. Phenol Red Mannitol Agar is used for differentiating pure cultures of bacteria based on mannitol fermentation reactions.

### Summary and Explanation

Phenol Red Agar Base with added carbohydrate is well suited for the study of fermentation reactions of microorganisms. However, while liquid media are generally employed in studying fermentation reactions, many bacteriologists prefer a solid medium for this purpose. One advantage of a solid fermentation medium is that it permits observation of fermentation reactions under both aerobic and anaerobic conditions. Deep tubes can provide sufficiently anaerobic conditions for the growth of obligate anaerobic bacilli. Any gas formation that occurs during a reaction is indicated by splitting of the agar or accumulation of gas bubbles in the base.

Phenol Red Agar Base supports excellent growth of many fastidious bacteria. It is a basal medium free of any fermentable carbohydrates that could give erroneous interpretations. With the exception of the omitted carbohydrate, it is a complete medium prepared with phenol red as an indicator of reaction changes. Phenol Red Agar Base permits the user to prepare any quantity of medium needed, adding to different portions any fermentable substance to be tested. Usually a final concentration of 0.5-1% of a test carbohydrate is added. An entire series of carbohydrate agars can be made up readily, conveniently and economically. Phenol Red Mannitol Agar already contains the specified carbohydrate.

### Principles of the Procedure

Peptone provides the carbon and nitrogen required for good growth of a wide variety of organisms. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Phenol red serves as a pH indicator, turning from red-orange to yellow when acid is produced during fermentation of the carbohydrate; if the carbohydrate is not fermented, the medium remains red or becomes alkaline (darker red).

### Formulae

**BBL™ Phenol Red Agar Base**

Approximate Formula* Per Liter

- Pancreatic Digest of Casein ....................................... 10.0 g
- Sodium Chloride ......................................................... 5.0 g
- Agar ......................................................................... 15.0 g
- Phenol Red................................................................ 18.0 mg

**Difco™ Phenol Red Mannitol Agar**

Approximate Formula* Per Liter

- Proteose Peptone No. 3 ................................. 10.0 g
- Beef Extract ................................................................. 1.0 g
- D-Mannitol ............................................................... 10.0 g
- Sodium Chloride ......................................................... 5.0 g
- Agar ......................................................................... 15.0 g
- Phenol Red............................................................... 25.0 mg

*Adjusted and/or supplemented as required to meet performance criteria.

### Directions for Preparation from Dehydrated Product

**BBL™ Phenol Red Agar Base**

1. Suspend 30 g of the powder in 1 L of purified water. Add carbohydrate, 5-10 g per L if desired. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. If addition of carbohydrate causes a fall in pH, readjust.
3. Dispense and autoclave at 118°C for 15 minutes. Alternatively, sterile carbohydrate solution may be added to cooled autoclaved solution.
4. Test samples of the finished product for performance using stable, typical control cultures.

**Difco™ Phenol Red Mannitol Agar**

1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.
**Identity Specifications**

**Difco™ Phenol Red Mannitol Agar**
- Dehydrated Appearance: Pink, free-flowing, homogeneous.
- Solution: 4.1% solution, soluble in purified water upon boiling. Solution is orange-red to red, very slightly opalescent.
- Prepared Appearance: Red to orange-red, slightly opalescent.
- Reaction of 4.1% Solution at 25°C: pH 7.4 ± 0.2

**BBL™ Phenol Red Agar Base**
- Dehydrated Appearance: Fine, homogeneous, without obvious foreign material.
- Solution: 3.0% solution, soluble in purified water upon boiling. Solution is medium to dark, red-orange to rose, clear to slightly hazy.
- Prepared Appearance: Medium to dark, red-orange to rose, clear to slightly hazy.
- Reaction of 3.0% Solution at 25°C: pH 7.4 ± 0.2

**Cultural Response**

**Difco™ Phenol Red Mannitol Agar**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>RECOVERY</th>
<th>ACID</th>
<th>GAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> subsp. enterica serotype Typhimurium</td>
<td>14028</td>
<td>Good</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>Good</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>9895</td>
<td>Good</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**BBL™ Phenol Red Agar Base**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>10^2-10^3</td>
<td>Good</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10145</td>
<td>10^2-10^3</td>
<td>Good</td>
</tr>
</tbody>
</table>

**Limitations of the Procedure**

1. The addition of some carbohydrates to the basal medium may cause an acid reaction. To restore the original pH (and color of the medium), add 0.1N sodium hydroxide on a drop-by-drop basis. Take care not to make the medium too alkaline, which would prevent fermentation from occurring within the usual incubation period.
2. When inoculating tubes, stab gently and do not use a loop. Rough stabbing or using a loop to stab may give the false appearance of gas production when mechanical splitting of the medium is what actually occurred.

**References**


**Availability**

**BBL™ Phenol Red Agar Base**
- Cat. No. 211502 Dehydrated – 500 g

**Difco™ Phenol Red Mannitol Agar**
- Cat. No. 210310 Dehydrated – 500 g