

# Phenol Red Broth Base

## Phenol Red Broth with Carbohydrates

### Intended Use

Phenol Red Broth Base and Phenol Red Broth with carbohydrates are used for the determination of fermentation reactions in the differentiation of microorganisms.

### Summary and Explanation

The ability of an organism to ferment a specific carbohydrate incorporated in a basal medium, resulting in the production of acid or acid and gas, has been used to characterize a specific species or group of bacteria, aid in the differentiation between genera and aid in species differentiation.<sup>1,2</sup>

In 1950, Vera recommended using pancreatic digest of casein in fermentation test media.<sup>3</sup> She found that casein peptone could be used with the pH indicator phenol red in fermentation tests with a high degree of accuracy.

Phenol Red Broth Base and Phenol Red Broth with Carbohydrates are referenced in the *Bacteriological Analytical Manual* for the differentiation of *Bacillus* and *Salmonella*.<sup>4</sup>

### Principles of the Procedure

Phenol Red Broth Base is a complete medium without added carbohydrate. It is used as a negative control for fermentation studies or as a base for the addition of carbohydrates by the aseptic addition of BBL™ Taxo™ Carbohydrate Discs. Pancreatic digest of casein provides nutrients and is low in fermentable carbohydrate.<sup>3</sup> The pH indicator, phenol red, is used to detect acid production.

Phenol Red Broths, prepared with a final concentration of one-half percent carbohydrate, are convenient for the determination of fermentation reactions. Most of the end products

### User Quality Control

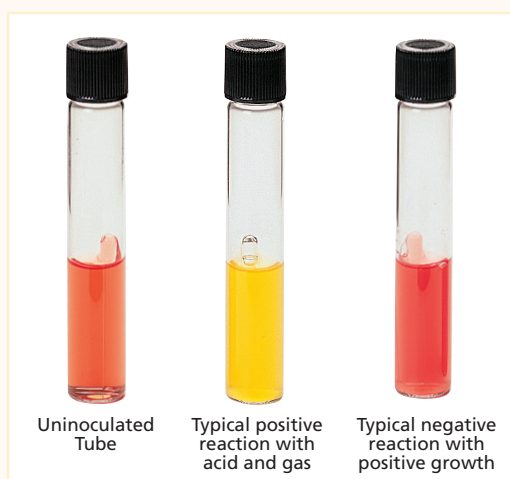
#### Identity Specifications

##### BBL™ Phenol Red Broth Base

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.  
 Solution: 1.5% solution, soluble in purified water. Solution is medium, orange-red to rose-red, clear to slightly hazy.  
 Prepared Appearance: Medium, orange-red to rose-red, clear to slightly hazy.  
 Reaction of 1.5% Solution at 25°C: pH 7.4 ± 0.2

##### BBL™ Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.  
 Solution: 2.0% solution, soluble in purified water. Solution is medium, orange-red to rose-red, clear to slightly hazy.  
 Prepared Appearance: Medium, orange-red to rose-red, clear to slightly hazy.  
 Reaction of 2.0% Solution at 25°C: pH 7.3 ± 0.2 (pH 7.4 ± 0.1 for Sucrose Broth)



#### Cultural Response

##### BBL™ Phenol Red Broth Base or Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	BASE	DEXTRROSE	LACTOSE	MANNITOL	SUCROSE
<i>Escherichia coli</i>	25922	K	AG	AG	AG	
<i>Enterococcus faecalis</i>	33186		A	A		A
<i>Proteus vulgaris</i>	8427	K	A		K	AG
<i>Pseudomonas aeruginosa</i>	10145	K	K			
<i>Salmonella Typhimurium*</i>	14028	K		K		K
<i>Shigella flexneri</i>	9199	K	A	K	A	K
<i>Staphylococcus aureus</i>	25923				A	

\**S. enterica* subsp. *enterica* serotype Typhimurium

KEY: A = growth with acid (yellow color)  
 K = growth with alkaline reaction (red color)  
 G = gas formation

For quality control organisms for prepared tubes of Phenol Red Broth with the various carbohydrates, consult the BBL™ Quality Control and Product Information Manual for Plated and Tubed Media.<sup>5</sup>

of carbohydrate fermentation are organic acids which, in the presence of phenol red, produce a color change in the medium from red to yellow.<sup>1</sup> If gas is produced during the fermentation reaction, it is collected in the inverted Durham tube.

No yellow color should occur in the control tube. If it does, the results cannot be correctly interpreted since acid has been produced without fermentation.

## Formulae

### BBL™ Phenol Red Broth Base

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	10.0 g
Sodium Chloride .....	5.0 g
Phenol Red.....	18.0 mg

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

### BBL™ Phenol Red Carbohydrate Broths

Contain the above ingredients with, per liter, 5.0 g of the specified carbohydrate.

## Directions for Preparation from Dehydrated Product

### BBL™ Phenol Red Broth Base

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Add carbohydrate (5-10 g), if desired. Agar (0.5-1.0 g) may be added if it is desirable to minimize convection currents in the broth. When agar is added, the medium should be boiled briefly.
3. Dispense in suitable tubes and insert Durham tubes when gas fermentation is to be recorded.
4. Autoclave at 118°C for 15 minutes. Tubes should be packed loosely to ensure free access of steam.
5. Alternatively, the base may be autoclaved and carbohydrates added aseptically as needed.
6. Test samples of the finished product for performance using stable, typical control cultures.

### BBL™ Phenol Red Broth with Dextrose or Lactose or Mannitol or Sucrose

1. Dissolve 20 g of the powder in 1 L of purified water. Agar (0.5-1.0 g) may be added if it is desirable to minimize convection currents in the broth. When agar is added, the medium should be boiled briefly.
2. Dispense and insert Durham tubes for detection of gas formation.
3. Autoclave at 118°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

If Taxo Carbohydrate Discs are being used with tubes of Phenol Red Broth Base, aseptically add the appropriate disc to the tubes prior to inoculation. Using a heavy inoculum, inoculate tubes of media with growth from an 18- to 24-hour pure culture using an inoculating loop. Incubate tubes with loosened caps at 35 ± 2°C for 18-48 hours either in an aerobic or anaerobic atmosphere depending on the organism being evaluated. Incubation up to 30 days may be necessary for a negative result.

## Expected Results

Examine the unsupplemented tubes at intervals during the incubation process for growth. If supplemented with carbohydrate, observe for the presence of acid (yellow color) and gas (as evidenced by displacement of the liquid in the Durham tubes).

Consult appropriate references for typical reactions produced by various microbial species.<sup>1,2,6-8</sup>

## References

1. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed., Lippincott Williams & Wilkins, Baltimore, Md.
2. Forbes, Sahn and Weissfeld. 2007. Diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Vera. 1950. Am. J. Public Health, 40:1267.
4. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
5. Becton, Dickinson and Co. 2007. BBL™ quality control and product information manual for plated and tubed media, BD Diagnostics, Sparks, Md.
6. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
7. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

## Availability

### BBL™ Phenol Red Broth Base

**AOAC BAM CCAM COMPF**

Cat. No. 211506 Dehydrated – 500 g  
221897 Prepared Tubes (K Tubes) – Pkg. of 10\*

### BBL™ Phenol Red Dextrose Broth

**BAM**

Cat. No. 211514 Dehydrated – 500 g

### BBL™ Phenol Red Lactose Broth

**BAM**

Cat. No. 211519 Dehydrated – 500 g

### BBL™ Phenol Red Mannitol Broth

**BAM**

Cat. No. 211527 Dehydrated – 500 g

### BBL™ Phenol Red Sucrose Broth

**BAM**

Cat. No. 211533 Dehydrated – 500 g

### BBL™ Phenol Red Broth with Carbohydrates and Durham Tube

**BAM**

Cat. No. 221671 Prepared Tubes with Adonitol – Pkg. of 10\*  
221673 Prepared Tubes with Arabinose – Pkg. of 10\*  
221675 Prepared Tubes with Cellobiose – Pkg. of 10\*  
221677 Prepared Tubes with Dextrose – Pkg. of 10\*  
221679 Prepared Tubes with Dulcitol – Pkg. of 10\*  
221681 Prepared Tubes with Glycerol – Pkg. of 10\*  
221683 Prepared Tubes with Inositol – Pkg. of 10\*  
221685 Prepared Tubes with Inulin – Pkg. of 10\*  
221687 Prepared Tubes with Lactose – Pkg. of 10\*  
221689 Prepared Tubes with Maltose – Pkg. of 10\*  
221691 Prepared Tubes with Mannitol – Pkg. of 10\*  
221693 Prepared Tubes with Raffinose – Pkg. of 10\*  
221695 Prepared Tubes with Rhamnose – Pkg. of 10\*  
221697 Prepared Tubes with Salicin – Pkg. of 10\*  
221699 Prepared Tubes with Sorbitol – Pkg. of 10\*  
221701 Prepared Tubes with Sucrose – Pkg. of 10\*  
221703 Prepared Tubes with Trehalose – Pkg. of 10\*  
221705 Prepared Tubes with Xylose – Pkg. of 10\*

\*Store at 2-8°C.