Phenol Red Agar Media
Phenol Red Agar Base • Phenol Red Mannitol Agar

Intended Use

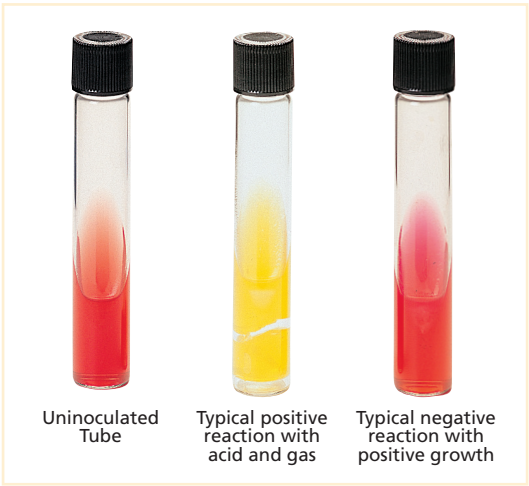
Phenol Red Agar Base is used with added carbohydrate in differentiating pure cultures of bacteria based on fermentation reactions.

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.[1-4] 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.



Uninoculated Tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

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.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

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

Cultural Response

Difco™ Phenol Red Mannitol Agar

Prepare the medium per label directions. Inoculate slant tubes with fresh cultures by stabbing the butt and streaking the slant surface. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	ACID	GAS
Escherichia coli	25922	Good	+	+
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Good	+	+
Staphylococcus aureus	25923	Good	+	–
Streptococcus mitis	9895	Good	–	–

Identity Specifications

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

BBL™ Phenol Red Agar Base

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 12-18 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Pseudomonas aeruginosa	10145	10^2-10^3	Good

Procedure

1. Inoculate the medium by stabbing into the butt and streaking the surface of the slant. If desired, inoculate obligate anaerobic bacteria into melted medium that has been cooled to 45°C. Allow the agar to solidify prior to incubation.
2. Incubate at 35 ± 2°C for 4-48 hours (or anaerobically for 24-72 hours).
3. Examine periodically for growth, acid production and gas formation.

Expected Results

Fermentation of the carbohydrate is indicated by a change in the color of the medium from red to canary yellow. Gas formation is indicated by the collection of gas bubbles in the base or by splitting of the agar.

For expected reactions with organisms on Phenol Red Agar Base supplemented with various carbohydrates, refer to appropriate references.[1-5]

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

1. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th edition. Mosby, Inc., St. Louis, Mo.
2. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th edition. American Society for Microbiology, Washington, D.C.
3. Holt, Krieg, Sneath, Staley and Williams. 1994. Bergey's Manual™ of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, N.Y.

Availability

BBL™ Phenol Red Agar Base

Cat. No. 211502 Dehydrated – 500 g

Difco™ Phenol Red Mannitol Agar

Cat. No. 210310 Dehydrated – 500 g