



PRODUCT SPECIFICATION SHEET

Phenol Red Raffinose Broth (DM308)

Intended Use

Phenol Red Raffinose Broth (DM308) is recommended for raffinose fermentation studies of microorganisms.

Product Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.^(1, 2) In 1950, Vera used a fermentation test medium employing the pH indicator phenol red and obtained accurate results.⁽³⁾ Phenol Red Broth Medium with various added carbohydrates serves as a differential medium by aiding in differentiation of various species and genera by their ability to ferment the specific carbohydrate, with the production of acid or acid and gas.⁽⁴⁻⁶⁾ Phenol Red Broth Medium is used as a negative control for studying fermentations or as a base for the addition of carbohydrates. Phenol Red Broth Base and Phenol Red Broth with Carbohydrates are referenced in the *Bacteriological Analytical Manual* for the differentiation of *Bacillus* and *Salmonella*. Phenol Red Raffinose Broth is used to study raffinose fermentation in various bacteria.

Principles of the Procedure

Phenol Red Broth Base contains proteose peptone and beef extract which provides the carbon and nitrogen required for good growth of a wide variety of organisms. Sodium chloride maintains the osmotic balance of the medium. Raffinose is the fermentable carbohydrate. Phenol red serves as a pH indicator. A positive raffinose fermentation reaction is indicated by the production of a yellow colour in broth due to the effect of acid production. Gas formation is seen in Durhams tubes. All of the *Enterobacteriaceae* grow well in this medium. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Raffinose	5.00
Phenol red	0.018
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 21 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Distribute in fermentation tubes (tubes containing inverted Durham's tubes).
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.





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Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Red coloured clear solution without any precipitate
Reaction of 2.1% solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms Preparation of Test Strain	Results to be achieved			
		Inoculum (CFU)	Growth	Acid	Gas
1.	<i>Citrobacter freundii</i> ATCC 8090	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
2.	<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
3.	<i>Enterobacter aerogenes</i> ATCC 3048	50-100	good-luxuriant	positive reaction, yellow colour	positive reaction
4.	<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	good-luxuriant	-	positive reaction
5.	<i>Proteus vulgaris</i> ATCC 13315	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
6.	<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
7.	<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
8.	<i>Serratia marcescens</i> ATCC 8100	50-100	good-luxuriant	negative reaction, no colour change	negative reaction
9.	<i>Shigella flexneri</i> ATCC 12022	50-100	good-luxuriant	negative reaction, no colour change	negative reaction

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Using a heavy inoculum, inoculate tubes of media with growth from an 18- to 24-hour old pure culture using an inoculating loop.
2. Incubate tubes with loosened caps at 35 - 37°C for 18-24 hours either in an aerobic or anaerobic atmosphere depending on the organism being evaluated.

Results

1. A yellow color in the medium indicates a positive reaction for carbohydrate fermentation.
2. If a Durham tube is used, bubbles in the inverted tube is an indication of gas production.
3. The presence of a single bubble is recorded as positive for the production of gas.
4. Refer to appropriate references for typical reactions produced by various microbial species.





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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. To ensure accuracy of interpretation, uninoculated control tubes and/or inoculated Phenol Red Broth Base control tubes should be run in parallel with the fermentation tests.

Packaging

Product Name : Phenol Red Raffinose Broth

Product Code : DM308

Available Pack sizes : 100gm

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.

Further Information

For further information please contact your local MICROMASTER Representative.



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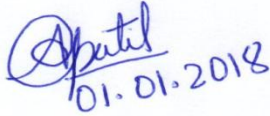

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