



PHENOL RED BROTH BASE (7148)

Intended Use

Phenol Red Broth Base is used with carbohydrates for the differentiation of microorganisms on the basis of carbohydrate fermentation reactions in a laboratory setting. Phenol Red Broth Base is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.^{1,2} A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting the growth of test organisms and free from fermentable carbohydrates. Vera used a fermentation test medium employing the pH indicator phenol red and obtained accurate results.³

Phenol Red Broth Base is recommended for use to determine the ability of organisms to ferment various carbohydrates.⁴⁻⁶ Various fermentable substances may be added in any desired concentration. The concentration of carbohydrate generally employed for testing fermentation reactions of bacteria is 0.5 to 1%. Some investigators prefer to use 1% rather than 0.5% to ensure against reversion of the reaction due to depletion of the carbohydrate.

Principles of the Procedure

The nitrogen, amino acids, and carbon sources are provided by Enzymatic Digest of Casein in Phenol Red Broth Base. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red serves as an indicator, turning from red-orange to yellow when acid is produced during fermentation of the added carbohydrates.

Formula / Liter

Enzymatic Digest of Casein 10 g
Sodium Chloride 5 g
Phenol Red 0.018 g

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

Desired Carbohydrate, 5 - 10 g

Precautions

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

Directions

1. Suspend 15 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. If desired, add carbohydrates (5 - 10 grams). Autoclave at 121°C for 15 minutes.
4. Alternatively, filtered sterilized carbohydrate solutions may be added to the cooled sterilized broth.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light reddish to pinkish-beige to white.

Prepared Appearance: Prepared medium without carbohydrates is bright red to red-orange and brilliant to clear, with or without light precipitate.

Expected Cultural Response: Cultural response in Phenol Red Broth Base incubated aerobically at 35 ± 2°C and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results W/ Dextrose		
		Growth	Acid	Gas
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good	+	+
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Good	-	-
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Good	+	+

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

Test Procedure

1. Inoculate tubes with isolated colonies.
2. An inverted Durham tube, added to the broth medium prior to sterilization, is used to detect gas production.
3. Incubate at $35 \pm 2^\circ\text{C}$ for 18 - 48 hours with loose caps.
4. Examine tubes for growth, acid production, and gas production (if Durham tube is used).

Results

A yellow color in the medium indicates a positive reaction for carbohydrate fermentation. If a Durham tube is used, bubbles in the inverted tube is an indication of gas production. The presence of a single bubble is recorded as positive for the production of gas.⁷

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

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

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. The addition of some carbohydrates to the basal medium may result in an acid reaction. 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

Phenol Red Broth Base	Code No.	7148A	500 g
		7148B	2 kg
		7148C	10 kg

References

1. **Isenberg, H. D. (ed.)**. 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.)**. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Vera, H. D.** 1950. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am. J. Public Health. **40**:1267.
4. **Bacteriological Analytical Manual**. 1995. 8th ed. AOAC International, Gaithersburg, MD.
5. **Vanderzant, C., and D. F. Splittstoesser**. 1992. Compendium of methods for the microbiological examination of food. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International. AOAC International, Arlington, VA.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.