Purple Broth Base

M284D

Purple Broth Base is recommended for the fermentation studies of *Listeria monocytogenes* .

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	1.000
Sodium chloride	5.000
Bromo cresol purple	0.020
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16.02 grams in 1000 ml distilled water. If desired add 5-10 grams of the carbohydrate to be tested. Heat if necessary to dissolve the medium completely. Dispense in tubes containing inverted Durhams tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Alternatively, to 900 ml of sterile and cooled basal medium aseptically add 100 ml of sterile 5 - 10% solution (final concentration 0.5 to 1 %)

Principle And Interpretation

Purple Broth Base is used for studying carbohydrate fermentation reactions, particularly in the identification of gram-negative enteric bacteria on addition of the desired carbohydrate (1, 2). Purple media were originally formulated by Vera (3) and further modified by addition of beef extract (4). These media are recommended by FDA (5) for fermentation studies of sugars. Purple Broth Base (M284D) differs from Purple Broth Base (M284) with the addition of beef extract in the former.

Beef extract and peptone special or proteose peptone supply the essential nutrients especially nitrogen sources to the growing organisms. Sodium chloride maintains the osmotic balance of the medium. Bromocresol purple is the pH indicator, which turns yellow at acidic pH. Gas production is evident by its collection in Durham's tube. The acid produced during the fermentation of carbohydrate causes bromocresol purple, the pH indicator to turn yellow. If the carbohydrate is not utilized or fermented, the color of the medium remains unchanged or becomes more alkaline (darker purple) due to decarboxylation of the amino acids present in the medium.

The broth is inoculated with 18 to 24 hours old pure culture and incubated at 35 ± 2°C for 24 to 72 hours (upto 30 days if necessary) either in an aerobic or anaerobic atmosphere depending on the organism being tested. It is recommended (6) to add carbohydrate in 1% concentration to avoid possible reversion reactions except glucose (dextrose). If the medium containing carbohydrate is sterilized by autoclaving, precautions should be taken to use minimum amount of heat required for sterilization to avoid hydrolysis of the carbohydrate.

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear solution in tubes

Reaction
Reaction of 1.6% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M284D: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with and without addition of 1% Dextrose

Organism	Inoculum (CFU)	Growth	Acid (without carbohydrate)	Gas (without carbohydrate)	Acid (with1% dextrose)	Gas (with1% dextrose)
Cultural Response						
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	positive reaction
Listeria monocytogenes ATCC 19112	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour (fermentative metabolism)	negative reaction
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	negative reaction
Staphylococcus aureus ATCC 25923	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Ewing W. H., 1986, Edwards and Ewings identification of Enterobacteriaceae , 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
2. Forbes B. A., Sahm A. S., and Weissfeld D. F., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc., St. Louis, Mo.
3. Vera H. D., 1950, Am. J. Public Health, 40:1267.
4. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
5. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. Wilkins, Baltimore and I Williams.

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