Purple Agar Base

M098

Intended Use:

Recommended for preparation of carbohydrate media used in fermentation studies for the cultural identification of pure cultures of enteric and other microorganims.

Composition**

Ingredients	Gms / Litre
Peptone special	10.000
HM peptone B #	1.000
Sodium chloride	5.000
Bromo cresol purple	0.020
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Meat extract B

Directions

Suspend 31.02 grams in 1000 ml purified / distilled water. Add 5 - 10 grams of the carbohydrate to be tested. Heat to boiling to dissolve the medium completely. Dispense in tubes as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Alternatively sterilize the basal medium prepared using 900 ml purified / distilled water and add 100 ml separately sterilized 5 - 10%solution of the desired carbohydrate to it.

Principle And Interpretation

Purple Agar Base is used for studying carbohydrate fermentation reactions, particularly in the identification of gram-negative enteric bacteria on addition of the desired carbohydrate (1, 4). Purple media were originally formulated by Vera (8) and further modified by addition of HM peptone B (3). These media are recommended by FDA (2) for fermentation studies of sugars.

HM peptone B and peptone special supply nitrogeneous and carbonaceous compounds, long chain amino acids and other 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 splitting of agar. 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. It is recommended (7) 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.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

For isolated Microorganisms samples follow appropriate techniques for handling specimens as per established guidelines (5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.It is recommended (6) to add carbohydrate in 1% concentration to avoid possible reversion reactions except glucose (dextrose).

2. 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.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple coloured clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Acid (without carbohydrate)	Gas (without carbohydrate)	Acid (with1% dextrose)	Gas (with1% dextrose)
Escherichia coli ATCC 25922 (00013*)	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 subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	negative reaction, no colour change	negative reaction	positive reaction, yellow colour	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

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3. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. Wilkins, Baltimore and I Williams.

8. Vera H. D., 1950, Am. J. Public Health, 40:1267.

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