



Folic Acid Culture Agar

M134

Intended Use:

Recommended for maintenance of *Enterococcus hirae* ATCC 8043.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Peptonized SM powder # | 15.000 |
| Yeast extract | 5.000 |
| Dextrose (Glucose) | 10.000 |
| Potassium dihydrogen phosphate | 2.000 |
| Tomato juice (100 ml) | 5.000 |
| Polysorbate 80 (Tween 80) | 1.000 |
| Agar | 10.000 |
| Final pH (at 25°C) | 6.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

Equivalent to Peptonized milk

Directions

Suspend 48 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Distribute in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes to 45-50°C rapidly in an upright position.

Principle And Interpretation

An important part of any assay is the maintenance and inoculum preparation of the test organism. Folic Acid Culture Agar is used to maintain stock cultures of a wide variety of test cultures like *Lactobacillus leichmannii* ATCC 7830, *Enterococcus hirae* ATCC 8043, *Lactobacillus plantarum (casei)* ATCC 8014 and *Lactobacillus rhamnosus* ATCC 7469 used in microbiological assay of vitamins.

Folic Acid Culture Agar is formulated as described by Kavanagh (3) and recommended by AOAC (4) for maintenance of *Enterococcus hirae* ATCC 8043, the test organism for Folic Acid Assay Medium (3).

Yeast extract and peptonized milk supply mainly the nitrogenous nutrients, vitamins and minerals essential for the growth of the test organisms. Dextrose is the energy source in the medium while tomato juice provides the growth factors. Polysorbate 80 maintains the surface tension of the medium to the optimal level while phosphate serves as buffering to the medium.

Inoculate 10 ml of Folic Acid Inoculum Medium (M541) with an 18-24 hours old culture from Folic Acid Culture Agar. Incubate at 35-37°C for 18-24 hours. Centrifuge the growth and resuspend the sediment in 10 ml of 0.85 % sterile saline, after decanting the supernatant. Repeat washing with saline, two more times. Dilute 1 ml of the washed cell suspension with 99 ml of 0.85% sterile saline (1:100). Adjust the inoculum concentration as per requirement or standard reference (4).

Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent free clean glassware should be used. Even small amount of contamination by foreign material can lead to erroneous results.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

For isolated microorganism samples follow appropriate techniques for handling specimens as per established guidelines (4). 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. Extreme care should be taken to avoid contamination of media or glassware used for the assay.
2. Detergent free clean glassware should be used.

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 yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 4.8% 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 18-24 hours.

| Organism | Inoculum (CFU) | Growth |
|---|----------------|-----------|
| # <i>Lactobacillus rhamnosus</i> ATCC 7469 (00101*) | 50-100 | luxuriant |
| <i>Lactobacillus leichmannii</i> ATCC 7830 | 50-100 | luxuriant |
| <i>Lactobacillus plantarum</i> ATCC 8014 | 50-100 | luxuriant |
| <i>Enterococcus hirae</i> ATCC 8043 (00089*) | 50-100 | luxuriant |

Key : (*) Corresponding WDCM numbers.

(#) Formerly known as *Lactobacillus casei*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Kavanagh F., 1963, Analytical Microbiology, Academic Press, New York.
4. Williams. (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., AOAC, Washington, D.C.

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Disclaimer :

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