Folic Acid Culture Agar

M134

Folic Acid Culture Agar is recommended for the maintenance of *Enterococcus hirae* ATCC 8043, which is used as a test organism in folic acid assay.

Composition**

Ingredients	Gms / Litre
Peptonized milk	15.000
Yeast extract	5.000
Dextrose	10.000
Monopotassium phosphate	2.000
Tomato juice (100 ml)	5.000
Polysorbate 80	1.000
Agar	10.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 48 grams in 1000 ml 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 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 (1) and recommended by AOAC (2) for maintenance of *Enterococcus hirae* ATCC 8043, the test organism for Folic Acid Assay Medium (1).

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 (2).

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.

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

M134: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
Lactobacillus casei ATCC 7469	50-100	luxuriant
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant
Enterococcus hirae ATCC 8043	50-100	luxuriant

Storage and Shelf Life

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

Reference

1.Kavanagh F., 1963, Analytical Microbiology, Academic Press, New York.
2.Williams. (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., AOAC, Washington, D.C.

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