



Folic Acid Inoculum Medium

M541

Folic Acid Inoculum Medium is recommended for the preparation of inoculum of *Enterococcus hirae* ATCC 8043 (formerly *Streptococcus faecium* ATCC 8043), which is used as a test organism for Folic Acid Assay Medium.

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
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

An important part of any assay is the maintenance and inoculum preparation of the test organism. Folic Acid Inoculum Medium is used for the preparation of inoculum to be used in the assay of the vitamins. Folic Acid Inoculum Medium is formulated as described by Kavanagh (1) and recommended by AOAC (2) for inoculum preparation 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 with an 18-24 hours old culture from Folic Acid Culture Agar (M134). 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

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 3.8% 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 .

Cultural Response

Organism	Inoculum (CFU)	Growth
Cultural Response		
<i>Lactobacillus casei</i> ATCC 7469	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	50-100	luxuriant

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. 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.

Revision : 2 / 2015

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