Folic AOAC Medium

Intended Use

Folic AOAC Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Folic AOAC Medium is prepared for use in the microbiological assay of folic acid according to the procedures of the Folic Acid Assay in the *Official Methods of Analysis of AOAC International*.[1] *Enterococcus hirae* ATCC™ 8043 is the test organism in this assay.

Principles of the Procedure

Folic Acid AOAC Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC 8043. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Folic AOAC Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	10.0	g
L-Asparagine	0.6	g
L-Tryptophan	0.2	g
L-Cysteine Hydrochloride	0.76	g
Dextrose	40.0	g
Adenine Sulfate	10.0	mg
Guanine Hydrochloride	10.0	mg
Uracil	10.0	mg
Xanthine	20.0	mg
p-Aminobenzoic Acid	1.0	mg
Pyridoxine Hydrochloride	4.0	mg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	800.0	µg
Nicotinic Acid	800.0	µg
Biotin	20.0	µg
Riboflavin	1.0	mg
Glutathione	5.2	mg
Polysorbate 80	0.1	g
Sodium Citrate	52.0	g
Dipotassium Phosphate	6.4	g
Magnesium Sulfate	0.4	g
Manganese Sulfate	20.0	mg
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 11 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Distribute 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

Procedure

Follow assay procedures as outlined in the reference.[1] It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that can influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 1, 2, 4, 6, 8 and 10 ng per assay tube (10 mL). Folic AOAC Medium may be used for both turbidimetric and titrimetric analysis. Turbidimetric readings should be taken after incubation at 35-37°C for 16-18 hours. Titrimetric determinations are best made following incubation at 35-37°C for 72 hours.

The folic acid required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried folic acid in about 30 mL 0.01N NaOH and 300 mL purified water.
B. Adjust the pH reaction to 7.5 ± 0.5 with diluted HCl solution.Dilute to 500 mL with purified water.
C. Add 2 mL of the solution to 50 mL purified water. Adjust the pH reaction to 7.5 ± 0.5. Dilute to 100 mL with purified water. This yields a stock solution containing 2 µg folic acid per mL.
D. Prepare the stock solution fresh daily.

The standard solution for the assay is made by diluting 1 mL of this stock solution to 1 liter with purified water. This solution contains 2 ng folic acid per mL. Use 0.0, 0.5, 1, 2, 3, 4, and 5 mL per assay tube.

Some laboratories may wish to alter the concentration of folic acid recommended above for the standard curve. This is permissible if the concentration used is within the limits specified by AOAC.[1]

User Quality Control

Identity Specifications

Difco™ Folic AOAC Medium

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	5.5% (single strength) or 11.0% (double strength) solution, soluble in purified water upon boiling. Single strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear.
Reaction of 5.5% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Folic AOAC Medium

Prepare the medium per label directions. The medium supports the growth of *Enterococcus hirae* ATCC™ 8043 when prepared in single strength and supplemented with folic acid. The medium should produce a standard curve when tested using a folic acid reference standard at 0.0 to 10.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-18 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

Reference

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC international, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Folic AOAC Medium

AOAC

Cat. No. 212169 Dehydrated – 100 g*

**Store at 2-8°C.*