Folic Acid Assay Medium

Intended Use

Folic Acid Assay 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 medium 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 Acid Assay Medium is used in the microbiological assay of folic acid with *Enterococcus hirae* ATCC™ 8043 as the test organism. Folic Acid Assay Medium is prepared according to the formula described by Capps, Hobbs and Fox,[1] modified with sodium citrate instead of sodium acetate.

Principles of the Procedure

Folic Acid Assay 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.

Formula

Difco™ Folic Acid Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Citrate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Biotin	0.8	µg
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese 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 sterilization and cooling conditions uniform throughout the assay.

User Quality Control

Identity Specifications

Difco™ Folic Acid Assay Medium

Dehydrated Appearance:	Off-white to very light beige, free-flowing, homogeneous.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in purified water upon boiling for 2-3 minutes. Single strength solution is light amber, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Folic Acid Assay 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 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Directions for Preparation from Dehydrated Product

1. Suspend 7.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense in 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 10 minutes.

Procedure

Prepare stock cultures of *E. hirae* ATCC 8043 by stab inoculation of Lactobacilli Agar AOAC. Incubate at 35-37°C for 24-48 hours. Store tubes in the refrigerator. Make transfers at monthly intervals. Prepare the inoculum for assay by subculturing a stock culture of *E. hirae* ATCC 8043 into a tube containing 10 mL of Lactobacilli Broth AOAC. After incubation at 35-37°C for 18-24 hours, centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 mL of sterile 0.85% saline. After the third wash, dilute the cell suspension 1:100 with sterile 0.85% saline. Use one drop of this latter suspension to inoculate each of the assay tubes.

It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 2, 4, 6, 8 and 10 ng per 10 mL assay tube. Turbidimetric readings should be made after incubation at 35-37°C for 18-24 hours. Refrigerate tubes for 15-30 minutes to stop growth before reading.

Prepare the folic acid stock solution required for the standard curve as follows:

1. Dissolve 50 mg dried Folic Acid USP Reference Standard or equivalent in about 30 mL of 0.01N NaOH and 300 mL purified water.
2. Adjust to pH 7.5 ± 0.5 with diluted HCl solution. Add purified water to give a volume of 500 mL.
3. Add 2 mL of the solution from step 2 to 50 mL purified water. Adjust the pH to 7.5 ± 0.5 with HCl solution. Dilute to 100 mL with purified water to give a stock solution containing 2 µg folic acid per mL. Prepare the stock solution fresh daily.

Prepare the standard solution for the assay by diluting 1 mL of this stock solution in 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.

Following incubation, place the tubes in the refrigerator for 15-30 minutes to stop growth. The growth can be measured by a turbidimetric method and the curve constructed from the values obtained. The most effective assay range is between the levels of 2 and 10 ng folic acid per 10 mL tube.

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. Capps, Hobbs and Fox. 1948. J. Bacteriol. *55*:869.

Availability

Difco™ Folic Acid Assay Medium

Cat. No. 231810 Dehydrated –100 g*

**Store at 2-8°C.*