Biotin Assay Medium

Intended Use

Biotin Assay Medium is used for determining biotin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are used 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.

Biotin Assay Medium is prepared for use in the microbiological assay of biotin using *Lactobacillus plantarum* ATCC™ 8014 as the test organism.

Principles of the Procedure

Biotin Assay Medium is a biotin-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC 8014. The addition of a biotin standard in specified increasing concentrations gives a growth response by this organism that can be measured titrimetrically or turbidimetrically.

User Quality Control

Identity Specifications

Difco™ Biotin Assay Medium

Dehydrated Appearance:	Light beige, homogeneous with a tendency to clump.
Solution:	3.75% (single strength) solution, soluble in purified water upon boiling 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Biotin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with biotin. The medium should produce a standard curve when tested with a biotin reference standard at 0.0 to 1.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-20 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Formula

Difco™ Biotin Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	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
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	2.0	mg
Pyridoxine Hydrochloride	4.0	mg
p-Aminobenzoic Acid	200.0	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.

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 to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

Procedure

Stock Cultures

Stock cultures of the test organism, *L. plantarum* ATCC 8014, are prepared by stab inoculation of Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the tubes are stored in the refrigerator. Transfers are made weekly.

Inoculum

Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC 8014 to 10 mL of single-strength Biotin Assay Medium supplemented with 0.5 ng biotin. After 16-24 hours incubation at 35-37°C, the cells are centrifuged

under aseptic conditions and the supernatant liquid decanted. The cells are washed three times with 10 mL sterile 0.85% saline. After the third wash, the cells are resuspended in 10 mL sterile 0.85% saline and finally diluted 1:100 with sterile 0.85% saline. One drop of this suspension is used to inoculate each 10 mL assay tube.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per assay tube (10 mL).

The concentration of biotin required for the preparation of the standard curve may be prepared by dissolving 0.1 gram of d-Biotin or equivalent in 1,000 mL of 25% alcohol solution (100 µg per mL). Dilute the stock solution by adding 2 mL to 98 mL of purified water. This solution is diluted by adding 1 mL to 999 mL purified water, giving a solution of 2 ng of biotin per mL. This solution is further diluted by adding 10 mL to 90 mL purified water, giving a final solution of 0.2 ng of biotin per mL. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 mL of this final solution. Prepare the stock solution fresh daily.

Biotin Assay Medium may be used for both turbidimetric and titrimetric analysis. Before reading, the tubes are refrigerated for 15-30 minutes to stop growth. Turbidimetric readings should be made after 16-20 hours at 35-37°C. Titrimetric determinations are made after 72 hours incubation at 35-37°C. The most effective assay range, using Biotin Assay Medium, has been found to be between 0.1 ng and 1 ng biotin.

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in the reference.[1]

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 by 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 to these procedures, all conditions of the assay must be followed precisely.

Reference

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Biotin Assay Medium

Cat. No. 241910 Dehydrated – 100 g*

**Store at 2-8°C.*