Pantothenate Assay Medium

Intended Use

Pantothenate Assay Medium is used for determining the concentration of pantothenic acid and its salts 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.

Pantothenate Assay Medium is a modification of the formula described in *The United States Pharmacopeia*[1] for the microbiological assay of calcium pantothenate using *Lactobacillus plantarum* ATCC™ 8014 as the test organism. Pantothenate Assay Medium does not contain polysorbate 80, which is included in **Difco**™ Pantothenate Medium AOAC.

Principles of the Procedure

Pantothenate Assay Medium is a dehydrated medium free from pantothenic acid or pantothenate but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Pantothenate Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	10.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	200.0	µg
Riboflavin	400.0	µg
Niacin	1.0	mg
Pyridoxine	800.0	µg
p-Aminobenzoic Acid	200.0	µg
Biotin	1.0	µg
Monopotassium Phosphate	1.0	g
Dipotassium 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 7.3 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 in 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 10 minutes.

Procedure

Prepare stock cultures of *L. plantarum* ATCC 8014 in triplicate by stab inoculation of Lactobacilli Agar AOAC. Incubate cultures for 18-24 hours at 35-37°C. Store the tubes at 2-8°C. Prepare a fresh stock culture every week. Do not use a culture older than 1 week for this assay.

Inoculum

Subculture from a stock culture of *Lactobacillus plantarum* ATCC 8014 to 10 mL of sterile single-strength Pantothenate Assay Medium supplemented with 0.02 µg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 mL sterile 0.85% saline. After the third wash, resuspend the cells with sterile 0.85% saline and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

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 readings and cannot always be duplicated.

User Quality Control

Identity Specifications

Difco™ Pantothenate Assay Medium

Dehydrated Appearance:	Very light beige, homogeneous with a tendency to clump.
Solution:	3.65% (single-strength) solution, soluble in purified water upon boiling for 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) light amber, clear, may have a slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Pantothenate 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 pantothenic acid. The medium should produce a standard curve when tested with a pantothenic acid reference standard at 0.0 to 0.10 µg 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.

The standard curve is obtained by using calcium pantothenate solution at levels of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08 and 0.1 µg per assay tube (10 mL). Turbidimetric determinations are made after 18-24 hours incubation at 35-37°C. Construct a standard curve and determine the concentration of the unknown by interpolation from the standard curve.

The concentration of pantothenic acid required for the preparation of the standard curve may be prepared by dissolving 50 mg dried calcium pantothenate in a solution containing approximately 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute to 1,150 mL with additional water to make the calcium pantothenate concentration 43.47 µg per mL; one mL equals 40 µg pantothenic acid.

This solution is diluted by adding 25 mL to a solution containing 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute to 1 liter with purified water to make a stock solution containing 1.0 µg pantothenic acid per mL. The standard solution is made by diluting 2 mL of the stock solution to 100 mL with purified water. This solution contains 0.02 µg pantothenic acid per mL. Use 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 mL per assay tube. Prepare the stock solution fresh daily.

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 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™ Pantothenate Assay Medium

Cat. No. 260410 Dehydrated – 100 g*

**Store at 2-8°C.*