Pantothenate Medium AOAC

Intended Use

Pantothenate Medium AOAC is used for determining the concentration of pantothenic acid and pantothenate by the microbiological assay technique.

Meets *United States Pharmacopeia (USP)* performance specifications.

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 Medium AOAC is prepared for use in the microbiological assay of pantothenic acid and pantothenate according to the procedures of Calcium Pantothenate Assay in the *USP*[1] and Pantothenate Acid Assay in the *Official Methods of Analysis of AOAC International* (AOAC).[2] *Lactobacillus plantarum* ATCC 8014 is the test organism used in this assay.

User Quality Control

Identity Specifications

Difco™ Pantothenate Medium AOAC

Dehydrated Appearance:	White to very light beige, homogeneous, tendency to clump.
Solution:	3.65% (single strength) or 7.3% (double strength) solution,soluble in purified water upon boiling for 2-3 minutes. Single-strength solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) light amber, clear, may have a very slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Pantothenate Medium AOAC

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 0.05 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.

Principles of the Procedure

Pantothenate Medium AOAC is a pantothenic acid/pantothenate-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *Lactobacillus 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 Medium AOAC

Approximate Formula* Per Liter

Dextrose	40.0	g
Sodium Acetate	20.0	g
Vitamin Assay Casamino Acids	10.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
L-Cystine	0.4	g
L-Tryptophan	0.1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Riboflavin	400.0	µg
Thiamine Hydrochloride	200.0	µg
Biotin	0.8	µg
p-Aminobenzoic Acid	200.0	µg
Nicotinic Acid	1.0	mg
Pyridoxine Hydrochloride	800.0	µg
Polysorbate 80	0.1	g

**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 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL.
6. Autoclave at 121°C for 10 minutes.

Procedure

Follow the assay procedures as outlined in *USP*[1] or AOAC.[2]

Prepare stock cultures of *L. plantarum* ATCC 8014 by stab inoculation of Lactobacilli Agar AOAC. Incubate stock cultures at 35-37°C (± 0.5°C) for 18-24 hours. Store the stock cultures at 2-8°C. Prepare fresh stab cultures every week. Do not use a culture more than one week old for preparing the inoculum.

Subculture from a stock culture of *Lactobacillus plantarum* ATCC 8014 to a tube of sterile single-strength Pantothenate Medium AOAC (10 mL) supplemented with 0.2 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% NaCl. After the third wash, resuspend the cells with sterile 0.85% NaCl 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.

Prepare solutions of Calcium Pantothenate USP Reference Standard or pantothenic acid (or equivalent) according to *USP*[1] or AOAC.[2] Satisfactory results are obtained with the standard curve by using pantothenic acid at levels of 0.0, 0.005, 0.01, 0.015, 0.02 and 0.025 µg per assay tube (10 mL) for the AOAC procedure. Calcium pantothenate may be used at standard levels of 0.0, 0.01, 0.02, 0.03, 0.04 and 0.05 µg per assay tube for the *USP* procedure. Pantothenate Medium AOAC may be used for both turbidimetric and titrimetric analysis in the AOAC procedure, and for turbidimetric analysis only for the *USP* procedure. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C (± 0.5°C). Titrimetric determinations are made following 72 hours incubation at 35-37°C (± 0.5°C).

The concentration of pantothenic acid or calcium pantothenate required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried calcium pantothenate in 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate.
2. Dilute with additional water to make calcium pantothenate concentration 43.47 µg per mL for the AOAC procedure or dilute to 50 µg per mL for the *USP* procedure. At 43.47 µg per mL, one mL should equal 40 µg pantothenic acid.

Dilute further by adding 25 mL of this solution to 500 mL purified water, 10 mL 0.2N acetic acid and 100 mL 0.2N sodium acetate. Dilute this solution to 1 liter with purified water to make a stock solution containing 1 µg pantothenic acid per mL. The standard solution is made by diluting 5 mL of the stock solution to 1000 mL with purified water to obtain a solution containing 0.005 µg pantothenic acid per mL. Use 0.0, 1, 2, 3, 4 and 5 mL per assay tube. For the *USP* procedure, dilute the 50 µg per mL solution with purified water to make a standard concentration of 0.01 µg per mL. Other standard concentrations may be used provided the standard falls within the limits specified by *USP*[1] and AOAC.[2]

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 and 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.

References

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

Availability

Difco™ Pantothenate Medium AOAC

AOAC USP

Cat. No. 281610 Dehydrated – 100 g*

**Store at 2-8°C.*