



Pantothenate Assay Medium

M037

Pantothenate Assay Medium is recommended for the microbiological assay of Pantothenic acid or its salts using *Lactobacillus plantarum* ATCC 8014 as the test organism.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate	10.000
Dextrose	40.000
Sodium acetate	20.000
L-Cystine	0.400
DL-Tryptophan	0.200
Adenine sulphate	0.020
Guanine hydrochloride	0.020
Uracil	0.020
Thiamine hydrochloride	0.0002
Riboflavin (Vitamin B2)	0.0004
Niacin	0.001
Pyridoxine	0.0008
p-Amino benzoic acid (PABA)	0.0002
Biotin	0.0000008
Monopotassium phosphate	1.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.400
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.020
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.31 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well to distribute the slight precipitate evenly. Dispense in 5 ml amounts to each assay tube in increasing amounts of the standard or the unknown and total volume 10 ml per tube is adjusted by addition of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Cool the medium immediately. Generally satisfactory results are obtained with Calcium pantothenate at levels of 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15 and 0.2 microgram per assay tube (10 ml).

Principle And Interpretation

Pantothenate Assay Medium is prepared according to the formulation of the U.S. Pharmacopeia (1) for microbiological assay of pantothenic acid or its salts using *Lactobacillus plantarum* ATCC 8014 as the test organism.

This medium contains several nutrients and salts which are required for the growth of the test organism. The nutrients include amino acids, carbohydrates, purine, pyrimidine bases, salts and vitamins but not the pantothenic acid. Since the pantothenate is required for the growth of *Lactobacillus plantarum* assay strain, growth of the organism will occur only if the materials being assayed contain pantothenate. Exact concentration of pantothenate in the test material can be calculated by comparing results with standard curve of pantothenate.

Assay / Procedure: Stock cultures of the test organism *Lactobacillus plantarum* ATCC 8014, are prepared in triplicate or more by stab inoculation of Pantothenate Culture Agar USP (M135). Following incubation for 16-24 hours at any selected temperature between 30°C and 37°C but held constant to within ± 0.5°C, the tubes are stored at 2-8°C. Prepare a fresh stab of stock culture every week and do not use a culture older than 1 week for transferring to broth for inoculation.

Inoculum for the assay is prepared by subculturing from a suitable stock culture of *Lactobacillus plantarum* ATCC 8014 on Pantothenate Culture Agar USP (M135) into a tube containing 10 ml of sterile single strength Pantothenate Assay Medium supplemented with pantothenate. The medium is prepared by dissolving 36.5 grams of the dehydrated medium and 20 mcg of pantothenate in 1000 ml of distilled water. Distribute in tubes and sterilize by autoclaving for 15 minutes at 15 lbs pressure (121°C). After 18-24 hours incubation at 30-37°C the cells are centrifuged under aseptic conditions and the supernatant liquid is decanted. The cells are resuspended in 10 ml sterile 0.85% sodium chloride. The cell suspension is then diluted 1:100 with sterile 0.85% sodium chloride. The cell suspension so obtained is the inoculum. Inoculate each tube aseptically with 1 drop of the inoculum.

It is essential that a standard curve be set up for each assay since conditions of autoclaving, temperature of incubation, etc. which influence the standard curve readings, cannot be duplicated exactly from time to time. The standard curve is obtained by using calcium pantothenate solution at concentration of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mcg pantothenic acid per assay tube (10 ml). Turbidimetric determinations are made after 16-24 hours incubation at any selected temperature between 30°C and 37°C, but held constant to within $\pm 0.5^\circ\text{C}$. Acidimetric determinations are made after 72 hours incubation at 30-37°C. A standard curve is then plotted and the unknown is determined by extrapolation.

Quality Control

Appearance

Off-white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution, which may have a slight precipitate.

Reaction

Reaction of 7.3% w/v aqueous solution at 25°C. pH : 6.8 \pm 0.2

pH

6.60-7.00

Cultural Response

M037: Microbiological Assay of Pantothenate is carried out by using *L.plantarum* ATCC 8014 after an incubation at 35-37°C for 18-24 hours.

Growth

Good growth is obtained. Gradually, increase in growth with increasing concentration of pantothenate standard level of 0.0, 0.025, 0.075, 0.1, 0.125, 0.15 and 0.2 mcg per assay tube is recorded as equivalent increase in absorbance at 620 nm.

Storage and Shelf Life

Store below 8°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1. U.S. Pharmacopeia, National Formulary, 2014, 37/NF 32, U.S. Pharmacopoeial Convention, Rockville, MD.

Revision : 2 / 2015

Disclaimer :

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