



## Pantothenate Assay Medium

M037

### Intended Use:

Recommended for microbiological assay of Pantothenic acid or its salts using *Lactobacillus plantarum* test organism.

### Composition\*\*

Ingredients	Gms / Litre
Acicase™	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

# Equivalent to Casein acid hydrolysate

### Directions

Suspend 7.31 grams in 100 ml purified/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 (3) 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  $\pm 0.5^\circ\text{C}$ , 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.

## Type of specimen

Pure isolates.

## Specimen Collection and Handling

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  $\pm 0.5^\circ\text{C}$ , 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.

## Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. Further biochemical testing is required for complete identification.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## 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

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 ingrowth 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 dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

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## Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. U.S. Pharmacopeia, National Formulary, 2002, 25/NF 20, U.S. Pharmacopoeial , Convention, Rockville, MD.

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### Disclaimer :

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