PRODUCT SPECIFICATION SHEET

Pantothenate Assay Medium (DM617)

Intended Use

Pantothenate Assay Medium (DM617) is recommended for microbiological assay of Pantothenic acid or its salts using *Lactobacillus plantarum ATCC 8014* as the test organism.

Product 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: Maintenance Media for carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose; Inoculum Media to condition the test culture for immediate use; and Assay Media to permit quantification 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 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.

Principles of the Procedure

Pantothenate Assay Medium is a dehydrated medium free from pantothenic acid or pantothenate but contains several nutrients including amino acids, carbohydrates, purine, pyrimidine bases, salts and vitamins which are required for the growth of *L. plantarum ATCC 8014*. 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.

Formula / Liter

Ingredients	Gms / Liter
Casein acid hydrolysate	10.00
Dextrose	40.00
Sodium acetate	20.00
L-Cystine	0.40
DL-Tryptophan	0.20
Adenine sulphate	0.02
Guanine hydrochloride	0.02
Uracil	0.02
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	1.00
Dipotassium phosphate	1.00
Magnesium sulphate	0.40
Sodium chloride	0.02
Ferrous sulphate	0.02
Manganese sulphate	0.02
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

PRODUCT SPECIFICATION SHEET

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. 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.
4. 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.
5. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions

1. Suspend 7.31 grams of medium in 100 ml of distilled water.
2. Heat to boiling to dissolve the medium completely. Mix well to distribute the slight precipitate evenly.
3. 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.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes.
5. Cool the medium immediately.
6. 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).

Quality Control Specifications

Dehydrated Appearance	Off-white to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear solution, which may have a slight precipitate
Reaction of 7.3% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected 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.

Test Procedure

a) Preparation of stock:

1. Stock cultures of the test organism *Lactobacillus plantarum ATCC 8014*, are prepared in triplicate or more by stab inoculation of Pantothenate Culture Agar USP (DM1191).
2. 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.

b) Inoculum:

1. Inoculum for the assay is prepared by subculturing from a suitable stock culture of *Lactobacillus plantarum ATCC 8014* on Pantothenate Culture Agar USP (DM1191) into a tube containing 10 ml of sterile single strength Pantothenate Assay Medium supplemented with pantothenate.
2. 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).
3. 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.
4. The cell suspension is then diluted 1:100 with sterile 0.85% sodium chloride. The cell suspension so obtained is the inoculum. Aseptically inoculate each assay tube with one drop of the cell suspension.

c) Standard Curve:

1. 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.

2. 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).
3. Turbidimetric determinations are made after 16-24 hours incubation at any selected temperature between 30°C and 37°C, but held constant to within ± 0.5°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.
4. 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.
5. 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.

Results

1. By plotting the response readings against the amount of standard in each tube, disk or cup, prepare a standard concentration response curve.
2. By interpolation from the standard curve, determine the amount of vitamin at each level of assay solution.
3. Calculate the concentration of vitamin 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%.
4. 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 equivalant increase in absorbance at 620 nm.
5. Refer to appropriate references and test procedures for interpretation of results.

Storage

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

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Pantothenate Assay Medium
Product Code : DM617
Available Pack sizes : 100gm

PRODUCT SPECIFICATION SHEET

References

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

Further Information

For further information please contact your local MICROMASTER Representative.

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