Lysine Assay Medium

M1932

It is recommended for determining lysine concentration by microbiological assay method.

Composition**

Ingredients	Gms / Litre
Dextrose	50.000
Sodium acetate	40.000
Ammonium chloride	6.000
Monopotassium phosphate	1.200
Dipotassium phosphate	1.200
Magnesium sulphate	0.400
Ferrous sulphate	0.020
Manganese sulphate	0.040
Sodium chloride	0.020
Adenine sulfate	0.020
Guanine hydrochloride	0.020
Uracil	0.020
Xanthine	0.020
Thiamine hydrochloride	0.001
Pyrodoxine hydrochloride	0.002
Pyridoxamine hydrochloride	0.600
Pyridoxal hydrochloride	0.600
Calcium panthothenate	0.001
Riboflavin	0.001
Nicotinic acid	0.002
p-Aminobenzoic acid	200.000mcg
Biotin	2.000mcg
Folic acid	20.000mcg
Glycine	0.200
DL-Alanine	0.400
Asparagine	0.800
L-Aspartic acid	0.200
L-Proline	0.200
DL-Serine	0.100
DL-Tryptophan	0.080
L-Glutamic acid	0.600
L-Histidine hydrochloride	0.124
DL-Phenylalanine	0.200
DL-Threonine	0.400
L-Tyrosine	0.200
DL-Valine	0.500
DL-Isoleucine	0.500
DL-Leucine	0.500
L-Arginine hydrochloride	0.484
L-Cystine	0.100
DL-Methionine	0.200
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.5 grams in 100 ml of purified 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.

Principle And Interpretation

Lysine Assay medium is formulated as described in Kavanagh (1). It contains all the essential growth factors for *Pediococcus acidilactici* ATCC 8042 except lysine. Exact concentration of of lysine in the test material can be calculated by comparing results with standard curve of lysine.

Assay/Procedure: Stock cultures of the test organism *Pediococcus acidilactici* ATCC 8042, are prepared by stab inoculation into Lacctobacilli Agar AOAC. Incubate the cultures at 35-37°C for 16-24 hours. After incubation, centrifuge aseptically and decant the supernatant. The pellet is washed 3-4 times with 10 ml of 0.85% NaCl solution. Then resuspend the cells in 10 ml of 0.85% NaCl solution. 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.

Increasing amounts of the standard or the unknown and make up the volume to 10ml per tube containing 5 ml of the rehydrated medium. The growth response is measured turbidometrically.

Quality Control

Appearance
Off-white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured clear solution, which may contain a slight precipitate.

Reaction
Reaction of 10.5% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M1932: Microbiological Assay of Lysine was carried out using Pediococcus acidilactici ATCC 8042 after an incubation at 35-37°C for 16-20 hours .

Organism	Growth
Cultural Response	
Pediococcus acidilactici ATCC 8042	Good growth is obtained. Gradual increase in growth with increasing conc.of standard L-Lysine 0, 30,60, 90, 120,150 mcg per assay tube was recorded as equivalent increase in absorbance at 660 nm.

Storage and Shelf Life

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

Reference

Kavanagh F., Analytical Microbiology Academic Press 1963, New York and London.

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