Methionine Assay Medium

M1937

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

Composition**

Ingredients	Gms / Litre
Sodium acetate	40.000
Ammonium chloride	6.000
Monopotassium phosphate	1.200
Dipotassium phosphate	1.200
Magnesium sulfate	0.400
Ferrous sulfate	0.020
Manganese sulfate	0.040
Sodium chloride	0.020
Adenine sulfate	0.020
Guanine hydrochloride	0.020
Uracil	0.020
Thiamine hydrochloride	0.001
Pyridoxine hydrochloride	0.002
Pyridoxamine hydrochloride	0.600
Pyrodoxal hydrochloride	0.600
Calcium panthothenate	0.001
Riboflavin	0.001
Nicotinic acid	0.002
p-Aminobenzoic acid	0.0002
Biotin	0.000002
Folic acid	0.00002
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
L-Lysine hydrochloride	0.500
Dextrose	50.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.62 grams of the dehydrated medium 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 15lbs pressure (121°C) for 10 minutes.

Principle And Interpretation

Pediococcus acidilactiti is gram positive organism and used as a probiotic. Methionine Assay Medium contains all the essential growth factors required for growth of *Pediococcus acididlactici* ATCC 8042 except L-Methionine. The addition of the amino acid in increasing concentrations gives growth response are prepared for use in the microbiological assay.

Three types of media used for the microbiological assay of amino acids are the maintenance media used for carrying the stock culture, the inoculum media for preparation of the inoculum and the assay media for quantitation of the amino acid (L-Methionine) under test.

Methionine Assay Medium is prepared as per the formulation of Steel et.al(1). It contains all other vitamins and nutrients essential for the growth of *Pediococcus acidilactici* ATCC 8042 except L-Methionine (amino acid under study).

Stock culture of *Pediococcus acidilactici* ATCC 8042 is prepared is prepared by stab inoculation into tubes of Lactobacilli Agar AOAC. The cultures are incubated at 35-37°C for 24 hours and stock cultures are maintained at 2-8°C. The inoculum is prepared by subculturing in 10 ml Lactobacilli Broth AOAC. Incubate at 35-37°C for 16-24 hours. After incubation, centrifuge the cells under aseptic conditions, decant the liquid supernatant. Wash the cells thrice with sterile 10 ml of sterile 0.85% NaCl solution. Then resuspend in 10 ml 0.85% NaCl solution. Dilute the solution as per use. The growth response obtained is turbidometrically or acidimetrically measured.

A standard curve is plotted with absorbance as a function of the L-methionine concentration. The concentration of L-methionine in the test sample is calculated based on the interpretation of the standard curve.

Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent-free clean glassware.

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 10.62% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH
6.50-6.90

Cultural Response
M1937: Microbiological Assay of L-Methionine 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- Methionine 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 mcg per assay tube was recorded as equivalent increase in absorbance at 660 nm.

Storage and Shelf Life

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

Reference

1.Steel,Sauberlich, Reynolds and Baumann 1949, J.Biol. Chem. 177:533

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Disclaimer :

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