Cystine Assay Medium

M1936

Intended Use:

Recommended for determining Cystine concentration by microbiological assay method.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	50.000
Sodium acetate	40.000
Ammonium chloride	6.000
Potassium dihydrogen phosphate	1.200
Dipotassium hydrogen phosphate	1.200
Magnesium sulphate	0.400
Ferrous sulphate	0.020
Manganese sulphate	0.040
Sodium chloride	0.020
Adenine sulphate	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 pantothenate	0.001
Riboflavin (Vitamin B2)	0.001
Nicotinic acid (Niacin)	0.002
p-Aminobenzoic acid (PABA)	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-Lysine hydrochloride	0.500
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 of the dehydrated medium in 100 ml of 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 purified / 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. Cystine Assay Medium contains all the essential growth factors required for growth of *Pediococcus acidilactiti* ATCC 8042 except L-Cystine. 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-Cystine) under test.

Cystine Assay Medium is prepared as per the formulation of Steel et.al (3). It contains all other vitamins and nutrients essential for the growth of *Pediococcus acidilactici* ATCC 8042 except L-cystine (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-cystine concentration. The concentration of L-cystine 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

Type of specimen

Isolated Microorganism

Specimen Collection and Handling

For isolated microorganism, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (3)

After use, contaminated materials must be sterilized by autoclaving before discarding.

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

NA

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 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

Microbiological Assay of Cystine was carried out using *Pediococcus acidilactici* ATCC 8042 after an incubation at 35-37°C for 16-20 hours .

Organism	Growth
Pediococcus acidilactici ATCC 8042	Good growth is obtained. Gradual increase in growth with increasing conc.of standard L-Cystine 0, 5, 10, 15, 20, 25 mcg per assay tube was recorded as equivalent increase in absorbance at 660 nm.

Storage and Shelf Life

Store dehydrated and 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

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

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