Amino Acid Assay Media
Lysine Assay Medium • Cystine Assay Medium

Intended Use

Lysine Assay Medium is used for determining lysine concentration by the microbiological assay technique.

Cystine Assay Medium is used for determining L-cystine concentration by the microbiological assay technique.

Summary and Explanation

Amino acid assay media are prepared for use in the microbiological assay of amino acids. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the amino acid under test. They contain all the factors necessary for optimal growth of the test organism except the single essential amino acid to be determined.

Amino Acid Assay Media are prepared according to the formulations of Steel et al.[1] They are used in the microbiological assay of amino acids using *Pediococcus acidilactici* ATCC™ 8042 as the test organism.

Principles of the Procedure

Lysine Assay Medium and Cystine Assay Medium contain all the factors essential for the growth of *Pediococcus acidilactici* ATCC 8042, except the amino acid under assay. The addition of the amino acid in specified increasing concentrations gives a growth response by the test organism.

Formulae[1]

Difco™ Lysine Assay Medium or Cystine Assay Medium

All amino acid assay media contain the following formula.

Approximate Formula* Per Liter		
Dextrose	50.0	g
Sodium Acetate	40.0	g
Ammonium Chloride	6.0	g
Monopotassium Phosphate	1.2	g
Dipotassium Phosphate	1.2	g
Magnesium Sulfate	0.4	g
Ferrous Sulfate	20.0	mg
Manganese Sulfate	40.0	mg
Sodium Chloride	20.0	mg
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Thiamine Hydrochloride	1.0	mg
Pyrodoxine Hydrochloride	2.0	mg
Pyridoxamine Hydrochloride	600.0	mg
Pyridoxal Hydrochloride	600.0	mg
Calcium Pantothenate	1.0	mg
Riboflavin	1.0	mg
Nicotinic Acid	2.0	mg
p-Aminobenzoic Acid	200.0	µg
Biotin	2.0	µg
Folic Acid	20.0	µg
Glycine	0.2	g
DL-Alanine	0.4	g
Asparagine	0.8	g
L-Aspartic Acid	0.2	g
L-Proline	0.2	g
DL-Serine	0.1	g
DL-Tryptophan	80.0	mg
L-Glutamic Acid	0.6	g
L-Histidine Hydrochloride	124.0	mg
DL-Phenylalanine	0.2	g
DL-Threonine	0.4	g
L-Tyrosine	0.2	g
DL-Valine	0.5	g
DL-Isoleucine	0.5	g
DL-Leucine	0.5	g
L-Arginine Hydrochloride	484.0	mg
DL-Methionine	0.2	g

User Quality Control

Identity Specifications

Difco™ Lysine Assay Medium or Cystine Assay Medium

Dehydrated Appearance:	White to off-white, homogeneous, may have a tendency to clump.
Solution:	5.25% (single strength) solution, soluble in purified water upon boiling. Solution is light to medium amber, clear, may have a slight precipitate.
Prepared Appearance:	Single strength—Light to medium amber, clear, may have a slight precipitate
Reaction of 5.25% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Lysine Assay Medium or Cystine Assay Medium

Prepare the medium per label directions. These media support the growth of *Pediococcus acidilactici* ATCC™ 8042 when prepared in single strength and supplemented with the appropriate amino acid. Lysine Assay Medium should produce a standard curve when tested with L-Lysine at 0.0 to 300 µg per 10 mL. Cystine Assay Medium should produce a standard curve when tested with L-Cystine at 0 to 50 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-20 hours. Read the percent transmittance at 660 nm.

Preparation of inoculum dilution, amino acid stock and working solution.

ASSAY MEDIUM	TEST CULTURE	PREPARATION OF INOCULUM DILUTION (CELL SUSPENSION + STERILE 0.85% NACl)	PREPARATION OF AMINO ACID STOCK SOLUTION (AMINO ACID + PURIFIED H_2O)		STANDARD WORKING SOLUTION (STOCK SOLUTION + PURIFIED H_2O)	VOLUME OF STANDARD WORKING SOLUTION (ML/10 ML TUBE)	FINAL AMINO ACID CONCENTRATION µG/10 ML
Lysine Assay Medium	*Pediococcus acidilactici* ATCC™ 8042	1 mL + 19 mL	L-lysine	6 g + 1,000 mL	1 mL + 99 mL	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 30, 60, 90, 120, 150, 180, 240, 300
Cystine Assay Medium	*Pediococcus acidilactici* ATCC™ 8042	1 mL + 19 mL	L-cystine	1 g + 100 mL + 1 mL HCl heated, then cooled, add up to 1,000 mL	1 mL + 99 mL	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 5, 10, 15, 20, 25, 30, 40, 50

In addition to the ingredients listed on the previous page, the media contain per liter*:

Lysine Assay Medium
L-Cystine.....0.1 g

Cystine Assay Medium
L-Lysine Hydrochloride.....0.5 g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

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. 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. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 10.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Stock Culture and Inoculum

Stock cultures of *Pediococcus acidilactici* ATCC 8042 are prepared by stab inoculation into tubes of Lactobacilli Agar AOAC or Micro Assay Culture Agar. Incubate cultures at 35-37°C for 24 hours. Store stock cultures at 2-8°C. Make transfers at monthly intervals in triplicate.

The inoculum for assay is prepared by subculturing the test organism into 10 mL Lactobacilli Broth AOAC or Micro Inoculum Broth. Incubate at 35-37°C for 16-24 hours. After incubation, centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3 times with 10 mL sterile 0.85% NaCl solution. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl solution. Dilute the 10 mL cell suspension with the appropriate amount of sterile 0.85% NaCl solution. (See the table under User Quality Control, Cultural Response.) One drop of the diluted inoculum suspension is used to inoculate each of the assay tubes.

Amino Acid Solution

Prepare stock solutions of each amino acid as described in the table under User Quality Control. Prepare the stock solutions fresh daily.

Increasing amounts of the standard or the unknown and sufficient purified water to give a total volume of 10 mL per tube are added to the tubes containing 5 mL of the rehydrated medium. The appropriate volumes of the standards and their final concentrations are listed in the table.

Measure the growth response turbidimetrically or titrimetrically. Turbidimetric readings are made after incubation at 35-37°C for 16-20 hours. Titrimetric readings are made after incubation at 35-37°C for 72 hours.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of amino acid at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of amino acid in the sample 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%.

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 of these procedures, all conditions of the assay must be followed precisely.

Reference

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

Availability

Difco™ Lysine Assay Medium

Cat. No. 242210 Dehydrated – 100 g*

Difco™ Cystine Assay Medium

Cat. No. 246710 Dehydrated – 100 g*

**Store at 2-8°C.*