# TM 2172 – LYSINE ASSAY MEDIUM

## INTENDED USE

For determining lysine concentration by microbiological assay method.

## PRODUCT SUMMARY AND EXPLANATION

Lysine Assay medium is formulated as described in Kavanagh. It contains all the essential growth factors for *Pediococcus acidilactici* ATCC 8042 except lysine. Exact concentration 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.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **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 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 |

## PRINCIPLE

This medium consists of amino acids which provide necessary nitrogenous nutrients for the organisms. Glucose is the fermentable carbohydrate source in the medium. Phosphate buffers the medium. Sodium chloride helps to maintain the osmotic balance in the medium. Sulphates present in the medium helps to provide ions to the medium.

## INSTRUCTION FOR USE

- Dissolve 10.5 grams in 100 ml 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 distilled / purified water.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 10 minutes.

## QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Off-white to yellow homogeneous free flowing powder.

**Appearance of prepared medium** : Light amber coloured clear solution, which may contain a slight precipitate.

**pH (at 25°C)** : 6.7 ± 0.2

## INTERPRETATION

Cultural characteristics observed after incubation.

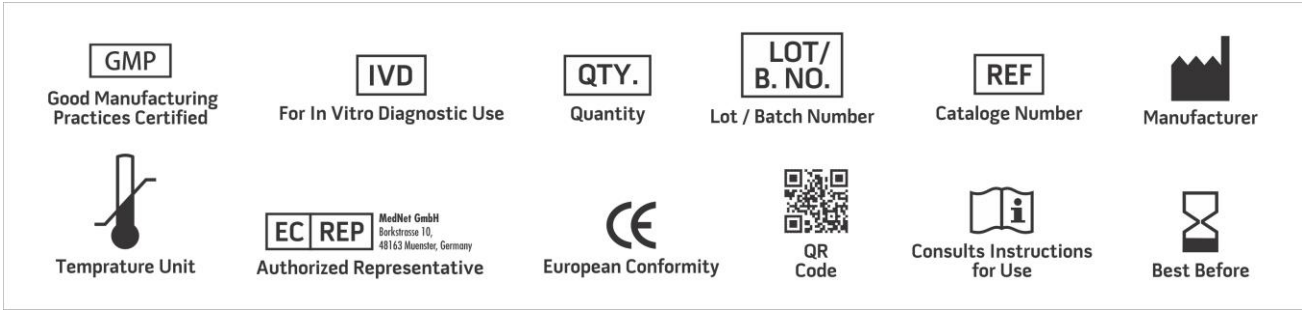
| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|
| *Pediococcus acidilactici* | 8042 | 50-100 | Good<br>Gradual increase in growth with increasing conc. of standard L-Lysine 0, 30,60, 90, 120,150 mcg per assay tube was recorded as equivalent increase in absorbance at 660 nm | 25-30°C | 24-48 Hours |

**PACKAGING:**
In pack size of 100 gm bottles.

**STORAGE**
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

**DISPOSAL**
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

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




**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
**Revision: 08 Nov., 2019**