

TM 172 – LYSINE MEDIUM BASE

INTENDED USE

For isolation and enumeration of wild yeasts in pitching yeasts.

PRODUCT SUMMARY AND EXPLANATION

Morris and Eddy described this complex medium for the isolation and enumeration of wild yeasts in pitching yeast in the brewery industry. Walters and Thiselton used a liquid synthetic medium containing lysine as sole nitrogen source and found that many types of yeast utilize lysine. Later Morris and Eddy also formulated solid lysine medium. Most of the *Saccharomyces* strains employed in the brewery industry and other fermentative industries do not use lysine, whereas the wild strains do.

Lysine Medium exploits this differential behavior to separate both types of yeasts. Morris and Eddy recommended surface inoculation of washed aliquots from the yeast mass; 0.2 ml suspension of 10⁷ cells/ml is the best. Sample is incubated at 25°C and examined daily, enumerating all the colonies that have grown (lysine positive). The degree of contamination is expressed as the number of wild yeast cells per million cells of the original inoculum. The number of cells in the inoculum is important as small number of cells about 100 to 1000 grow to a limited extent while 10,000 brewing yeast cells provide a direct measure of contaminant wild yeasts.

COMPOSITION

| Ingredients | Gms / Ltr |
|--------------------------------|-----------|
| Dextrose (Glucose) | 44.500 |
| Potassium dihydrogen phosphate | 1.780 |
| Magnesium sulphate | 0.890 |
| Calcium chloride anhydrous | 0.178 |
| Sodium chloride | 0.089 |
| Adenine | 0.00178 |
| DL-Methionine | 0.000891 |
| L-Histidine | 0.000891 |
| DL-Tryptophan | 0.000891 |
| Boric acid | 0.0000089 |
| Zinc sulphate | 0.0000356 |
| Ammonium molybdate | 0.0000178 |
| Manganese sulphate | 0.0000356 |
| Ferrous sulphate | 0.0002225 |
| L-Lysine | 1.000 |
| Inositol | 0.020 |
| Calcium pantothenate | 0.002 |



| | |
|-----------------------------|----------|
| Aneurine | 0.0004 |
| Pyridoxine | 0.0004 |
| p-Amino benzoic acid (PABA) | 0.0002 |
| Nicotinic acid (Niacin) | 0.0004 |
| Riboflavin (Vitamin B2) | 0.0002 |
| Biotin | 0.000002 |
| Folic acid | 0.000001 |
| Agar | 17.800 |

PRINCIPLE

This medium consists of vitamins and trace elements, which is necessary to support metabolic activities of yeast. Lysine acts as the sole source of nitrogen, which is utilized by many types of yeast. Dextrose 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 6.62 grams in 100 ml purified/distilled water containing 1 ml of 50% potassium lactate.
- Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE.
- Cool to 45-50°C, adjust pH to 5.0 with 10% lactic acid and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Whitish to Creamish, clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) : 5.0 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Incubation Temperature | Incubation Period |
|--------------------------|-------|-------------------|-----------|----------|------------------------|-------------------|
| <i>Pichia fermentans</i> | 10651 | 50-100 | Luxuriant | ≥70% | 25-30°C | Upto 7 Days |

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°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. Fowell R. R., 1965, J. Appl. Bacteriol., 28:373.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. Morris E. O. and Eddy A. A, 1957, J. Inst. Brew. 63(1): 34.
5. Walters L. S. and Thiselton M. R., 1953, J. Inst. Brew. 59:401.

| | | | | |
|--------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
|  Good Manufacturing Practices Certified |  Best Before |  Quantity |  Catalogue Number |  Manufacturer |
|  Temperature Unit |  Lot / Batch Number |  Consults Instructions for Use |  QR Code | |

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
Revision: 28 Aug., 2023