# MRS Agar, Modified

**M1990I**

## Intended Use:

Recommended isolation and enumeration of mesophilic lactic acid bacteria from food. The composition and performance criteria are in accordance with in ISO 15214:1998 and ISO 11133 : 2014 (E) /Amd. :2020.

## Composition**

**ISO specification - MRS Agar, Modified**

| Ingredients | g / L |
|---|---|
| Tryptone $ | 10.000 |
| HM extract ⊖ | 10.000 |
| Yeast extract | 4.000 |
| Triammonium citrate | 2.000 |
| Sodium acetate | 5.000 |
| Magnesium sulphate.7H2O | 0.200 |
| Manganese sulfate. 4H2O | 0.050 |
| Dipotassium hydrogen phosphate | 2.000 |
| Dextrose (Glucose) | 20.000 |
| Polysorbate 80 (Tween 80) | 1.080 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 5.7±0.1 |

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**Formula adjusted, standardized to suit performance parameters.

$ -Equivalent to Enzymatic digest of casein ⊖ -Equivalent to Meat extract

## Directions

Suspend 69.21 gram (equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

## Principle And Interpretation

MRS Agar, Modified is in accordance with ISO (1,2) for the enumeration of mesophilic lactic acid bacteria. Mesophilic bacteria are divided into two groups: Lactic Acid Starter bacteria (including *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris* ), which are primarily used for producing lactic acid, and Aroma Producing bacteria (including *Lactococcus lactis subsp. lactis biovar diacetylactis* and *Leuconostoc mesenteriodes subsp. cremoris*), which are primarily used for producing CO2 gas and flavor. MRS Agar, Modified (Lactobacillus Heteroferm Screen Agar) recommended by APHA (3), is used for the isolation and cultivation of *Lactobacillus* species from salad dressings (4). Tryptone, HM extract and yeast extract supply nitrogen, carbon and other elements essential for the growth of Lactobacilli. Dextrose (Glucose) is the carbohydrate source. Polysorbate 80 (Tween 80) supplies fatty acids required by Lactobacilli. Triammonium citrate, sodium acetate inhibit gram-negative organisms and certain gram-positive bacteria.

## Type of specimen

Food and animal feeding stuff

## Specimen Collection and Handling:

For food and animal feeding stuff samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,3).

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

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

## 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**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Yellow coloured slight opalescent gel forms in Petri plates

**Reaction**

Reaction of 6.92% w/v aqueous solution at 25°C. pH : 5.7±0.1

**pH**

5.60-5.80

**Cultural Response**

**Productivity :** Cultural characteristics observed after an incubation at 30 ± 1°C for 72 ± 3 hours . Recovery rate is considered as 100% for bacteria growth on Reference Medium - previously validated MRS Agar.

**Selectivity** : Cultural characteristics observed after an incubation at 30 ± 1°C for 72 ± 3 hours .

| Organism | Inoculum (CFU) | Growth | Recovery |
|---|---|---|---|
| **Productivity** | | | |
| *Lactobacillus sakei ATCC* 15521 (00015*) | 50-100 | good-luxuriant | >=70% |
| *Lactococcus lactis ATCC* 19435 00016*) | 50-100 | good-luxuriant | >=70% |
| *Pediococcus pentosaceus* ATCC 33316 (00158*) | 50-100 | good-luxuriant | >=70% |
| **Selectivity** | | | |
| *Escherichia coli ATCC 8739 (00012*)* | >=$10^4$ | inhibited | |
| *Escherichia coli ATCC* 25922 (00013*) | >=$10^4$ | inhibited | |
| *Bacillus cereus ATCC* 11778 (00001*) | >=$10^4$ | inhibited | |

Key : (*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store dehydrated and the 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 (5,6).

## References

1. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lactic acid bacteria. ISO 15214:1998(E).
2. Microbiology of food,animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014 (E) /Amd. :2020.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
4. Smittle R. B. and Flowers R. M., 1982, J. Food Protection, 45:977.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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.

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