

# **Technical Data**

# MRS Agar w/ Low pH

# **Intended Use:**

For cultivation of all Lactobacillus species from all types of material.

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Composition**	
Ingredients	<b>g</b> / L
HM peptone #	10.000
HM peptone B ##	10.000
Yeast extract	5.000
Diammonium hydrogen citrate	2.000
Dipotassium hydrogen phosphate	2.000
Dextrose (Glucose)	20.000
Magnesium sulphate heptahydrate	0.200
Manganese sulphate tetrahydrate	0.050
Sodium acetate trihydrate	5.000
Agar	12.000
Final pH ( at 25°C)	5.4±0.2

\*\*Formula adjusted, standardized to suit performance parameters

# - Equivalent to Meat peptone ## - Equivalent to Beef extract

## Directions

Suspend 64.15 (the equivalent weight of dehydrated medium per litre) grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. 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**

Lactobacilli MRS medium is based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from dairy products (2), foods (3) and other sources (4).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source.Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Magnesium sulphate and manganese sulphate provide essential ions for multiplication of lactobacilli. Phosphates provide good buffering action in the media

Lactobacilli are microaerophillic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate (4). Lactobacilli isolated on MRS Agar should be further confirmed biochemically.

# **Type of specimen**

Clinical sample-faeces ; Food and dairy samples.

# **Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,7,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

# **Warning and Precautions**

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

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## **Limitations :**

Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
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. Further biochemical and serological test must be carried out for further identification.

# **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 light yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.2% Agar gel.

#### Colour and Clarity of prepared medium

Medium to dark amber coloured, clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 6.42% w/v aqueous solution at  $25^{\circ}$ C. pH :  $5.4\pm0.2$ 

#### pН

5.20-5.60

#### **Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer (with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
<i>Lactobacillus casei</i> ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant	>=50%
<i>Lactobacillus plantarum</i> ATCC 8014	50-100	luxuriant	>=50%

# **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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

# Reference

- 1. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
- 2. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
- 3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
- 4. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
- 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.

7.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

8.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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