

Lactobacilli MRS Agar (NCM0035)

Intended Use

Lactobacilli MRS Agar is used for the cultivation of lactobacilli and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

MRS Agar is a medium for the cultivation and enumeration of *Lactobacillus* spp. Originally developed in 1960 by de Man, Rogosa & Sharpe, the medium is suitable for most lactic acid bacteria and is intended as a substitute for Tomato Juice Agar. When acidified to pH 5.4 MRS Agar can be used to enumerate *Lactobacillus bulgaricus* in yogurts.

Nutrition is provided by a mixture of carefully selected peptones, glucose, beef & yeast extracts while Tween® 80, magnesium and manganese sulfates act as growth stimulants. Selectivity against streptococci & molds is provided by ammonium citrate and sodium acetate. Used at low pH, ammonium citrate allows growth of lactobacilli while inhibiting a number of other microorganism groups. Occasionally, sterilization of this medium at 121°C for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 6.4 +/- 0.2. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

Typical Formulation

Peptone	10.0 g/L
Yeast Extract	5.0 g/L
Beef Extract	10.0 g/L
Glucose	20.0 g/L
Dipotassium Phosphate	2.0 g/L
Sodium Acetate	5.0 g/L
Triammonium Citrate	2.0 g/L
Magnesium Sulfate	0.2 g/L
Manganese Sulfate	0.05 g/L
Tween® 80	1.08 g/L
Agar	15.0 g/L

Final pH: 6.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 70 grams of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.
5. If acidified medium is required, adjust pH aseptically prior to pouring.

Test Procedure

1. To obtain direct counts of lactobacilli, pour 15 - 20 mL sterile, molten (45 - 50°C) Lactobacilli MRS Agar into sterile petri dishes containing 1 mL volumes of diluted test sample.
2. Distribute inoculum throughout medium by rotating the plate in one direction, then in the reverse direction.
3. Allow medium to solidify on a flat surface for 5 - 10 minutes.



- Alternatively, plates of Lactobacilli MRS Agar can be used for direct recovery of organisms using the streak inoculation technique.
- Incubate agar plates at 35°C for 3 days, or at 30°C for 5 days in an aerobic atmosphere supplemented with carbon dioxide.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous with slightly clumped and beige.

Prepared Appearance: Prepared medium is clear, no precipitate and amber.

Expected Cultural Response: Cultural response on Lactobacillus MRS Agar incubated aerobically at 30 ± 1°C and examined for growth after 48-72hrs.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Lactobacillus casei</i> ATCC® 393	50-200	>70%
<i>Lactobacillus delbrueckii</i> ATCC® 4797	50-200	>70%
<i>Lactobacillus rhamnosus</i> ATCC® 7469	50-200	>70%
<i>Lactobacillus fermentum</i> ATCC® 9338	4 Quad	Growth
<i>Lactobacillus parabuchneri</i> ATCC® 11307	4 Quad	Growth
<i>Lactobacillus plantarum</i> ATCC® 8014	50-200	>70%

The organisms listed are the minimum that should be used for quality control testing.

Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar. Growth can be sub-cultured onto appropriate media for use in additional procedures. Refer to appropriate references for recommendation on the identification of *Lactobacillus* spp.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

- Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.
- Organisms other than lactobacilli may grow in this medium. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

- de Man, J.C., Rogosa, M and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23, 130-135.
- MacFaddin, J. F. 1985. Media for the isolation-cultivation-identification-maintenance of medical bacteria, vol. 1 Williams & Wilkins, Baltimore, MD.
- Vanderzant, C. and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Technical Specification Sheet



4. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

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