

Lactobacilli MRS Agar • Lactobacilli MRS Broth

Intended Use

Lactobacilli MRS Agar and Lactobacilli MRS Broth are recommended for use in the isolation, enumeration and cultivation of *Lactobacillus* species.

Summary and Explanation

Lactobacilli MRS Agar and Lactobacilli MRS Broth are based on the formulations of deMan, Rogosa and Sharpe.¹ These media were shown by the authors to support luxuriant growth of all lactobacilli from oral, fecal, dairy and other sources.

Principles of the Procedure

Lactobacilli MRS Agar and Lactobacilli MRS Broth contain peptone and dextrose. These ingredients supply nitrogen, carbon and other elements necessary for growth. Polysorbate 80, acetate, magnesium and manganese provide growth factors for culturing a variety of lactobacilli. The above ingredients may inhibit the growth of some organisms other than lactobacilli.

Formulae

Difco™ Lactobacilli MRS Agar

| | |
|--------------------------------|--------|
| Approximate Formula* Per Liter | |
| Proteose Peptone No. 3..... | 10.0 g |
| Beef Extract..... | 10.0 g |
| Yeast Extract..... | 5.0 g |
| Dextrose..... | 20.0 g |
| Polysorbate 80..... | 1.0 g |
| Ammonium Citrate..... | 2.0 g |
| Sodium Acetate..... | 5.0 g |
| Magnesium Sulfate..... | 0.1 g |
| Manganese Sulfate..... | 0.05 g |
| Dipotassium Phosphate..... | 2.0 g |
| Agar..... | 15.0 g |

Difco™ Lactobacilli MRS Broth

Consists of the same ingredients without the agar.

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water.
 - Difco™ Lactobacilli MRS Agar – 70 g;
 - Difco™ Lactobacilli MRS Broth – 55 g.
 Mix thoroughly.

User Quality Control

Identity Specifications

Difco™ Lactobacilli MRS Agar

| | |
|------------------------------------|--|
| Dehydrated Appearance: | Light tan, free-flowing, homogeneous. |
| Solution: | 7.0% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent. |
| Prepared Appearance: | Medium amber, very slightly to slightly opalescent. |
| Reaction of 7.0% Solution at 25°C: | pH 6.5 ± 0.2 |

Difco™ Lactobacilli MRS Broth

| | |
|------------------------------------|---|
| Dehydrated Appearance: | Tan, homogeneous, appears moist. |
| Solution: | 5.5% solution, soluble in purified water upon boiling. Solution is medium amber, clear to very slightly opalescent. |
| Prepared Appearance: | Medium amber, clear to very slightly opalescent. |
| Reaction of 5.5% Solution at 25°C: | pH 6.5 ± 0.2 |

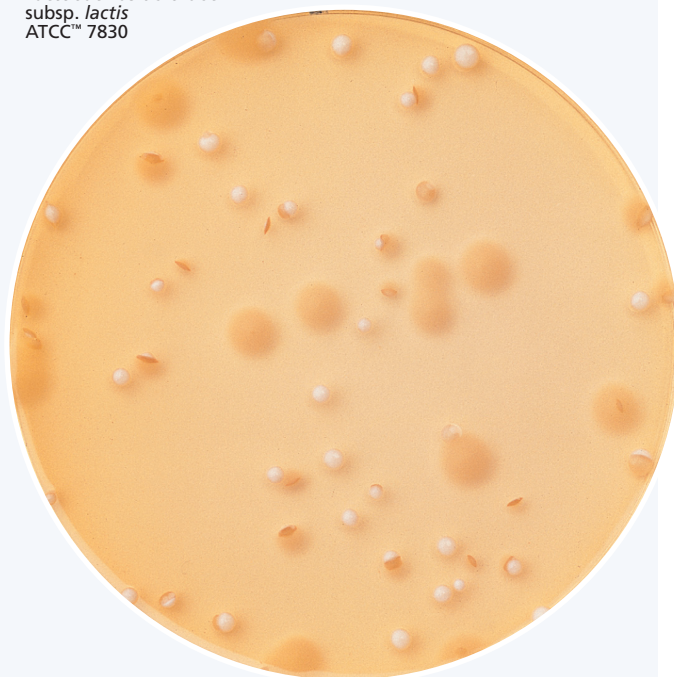
Cultural Response

Difco™ Lactobacilli MRS Agar or Lactobacilli MRS Broth

Prepare the medium per label directions. Inoculate Lactobacilli MRS Agar and incubate in a 5% CO₂ atmosphere at 35° ± 2°C for 24- 72 hours. Inoculate Lactobacilli MRS Broth and incubate at 35° ± 2°C for 18-24 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|---|-------|----------------------------------|----------|
| <i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> | 7830 | 10 ² -10 ³ | Good |
| <i>Lactobacillus fermentum</i> | 9338 | 10 ² -10 ³ | Good |
| <i>Lactobacillus johnsonii</i> | 11506 | 10 ² -10 ³ | Good |

Lactobacillus delbrueckii
subsp. *lactis*
ATCC™ 7830



- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Direct Counts

- 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.
- Distribute the inoculum throughout the medium by rotating the plate in one direction and then in the reverse direction.
- Allow the 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.

Broth Enrichment

- Samples can be inoculated directly into Lactobacilli MRS Broth.
- Incubate broth tubes at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere.
- Subculture growth in broth tubes to appropriate solid media.

Expected Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar or as turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.^{2,3}

Limitation of the Procedure

Organisms other than lactobacilli may grow in these media. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

References

- deMan, Rogosa and Sharpe. 1960. J. Appl. Bacteriol. 23:130.
- Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
- Downes and Ito(ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Lactobacilli MRS Agar

COMPF **ISO** **SMD**

Cat. No. 288210 Dehydrated – 500 g*

Difco™ Lactobacilli MRS Broth

COMPF **SMD**

Cat. No. 288130 Dehydrated – 500 g*
 288110 Dehydrated – 2 kg*
 288120 Dehydrated – 10 kg*

*Store at 2-8°C.