

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

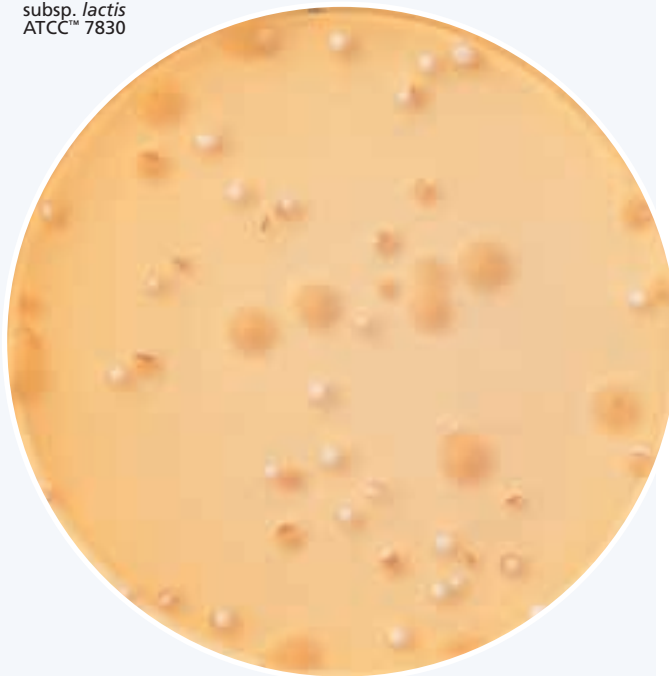
Cultural Response

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



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L Lactobacilli MRS Agar, cont.

- 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.

Lactose Broth

Intended Use

Lactose Broth is used for detection of the presence of coliform organisms, as a pre-enrichment broth for salmonellae and in the study of lactose fermentation of bacteria in general.

Summary and Explanation

Lactose Broth was formulated in accordance with recommendations of the American Public Health Association (APHA) and the American Water Works Association for testing dairy products and water for the presence of coliform organisms.^{1,2} This medium was, but no longer is, listed as an alternative to Lauryl Sulfate Broth in the presumptive portion of the Standard Total Coliform Multiple-Tube (MPN) Test for water analysis provided that it had been demonstrated not to increase the frequency of false-positives nor mask coliforms.³ It is one of the recommended media in the *Compendium of Methods for the Microbiological Examination of Foods* for pre-enrichment when *Salmonella* organisms are suspected in foods.⁴

Principles of the Procedure

The peptone and beef extract provide essential nutrients for bacterial metabolism. Lactose provides a source of fermentable carbohydrate for coliform organisms. Growth with the formation of gas is a presumptive test for coliforms.

Formula

Difco™ Lactose Broth

Approximate Formula* Per Liter

Beef Extract.....	3.0	g
Peptone	5.0	g
Lactose	5.0	g

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

Directions for Preparation from Dehydrated Product

- Suspend 13 g of the powder in 1 L of purified water. Mix thoroughly.
- Warm gently until solution is complete.
- Dispense in test tubes containing inverted Durham tubes, in 10 mL amounts for testing samples of 1 mL or less. For testing 10 mL quantities of samples, dissolve 26 g of the powder in