

# PRODUCT SPECIFICATION SHEET

## Lactobacillus MRS Agar (MRS Agar) (DM140)

### Intended Use

Lactobacillus MRS Agar (MRS Agar) (DM140) is recommended for isolation and cultivation of *Lactobacilli*.

### Product Summary and Explanation

*Lactobacilli* are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid. In humans they are present in the vagina and the gastrointestinal tract, where they make up a small portion of the gut flora.<sup>(1)</sup> They are usually benign, except in the mouth where they have been associated with cavities and tooth decay (dental caries). The production of lactic acid makes its environment acidic, which inhibits the growth of some harmful bacteria.

Lactobacilli MRS Agar is based on the formulation of deMan, Rogosa and Sharpe<sup>(2)</sup> with slight modification. This medium supports luxuriant growth of all *Lactobacilli* from oral cavity,<sup>(2)</sup> dairy products,<sup>(3)</sup> foods,<sup>(4)</sup> faeces<sup>(5)</sup> and other sources.<sup>(6)</sup>

### Principles of the Procedure

Lactobacilli MRS Agar contains proteose peptone and beef extract supply nitrogen, carbon and other nutrients necessary for growth of microorganisms. Yeast extract provides vitamin B complex. Dextrose is the fermentable carbohydrate and an energy source. Polysorbate 80 contributes to 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*. Dipotassium phosphate provides good buffering action in the media.

### Formula / Liter

Ingredients	Gms / Liter
<b>Part A</b>	--
Proteose peptone	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Ammonium citrate	2.00
Sodium acetate	5.00
Magnesium sulphate	0.10
Manganese sulphate	0.05
Dipotassium phosphate	2.00
Agar	12.00
<b>Part B</b>	--
Polysorbate 80	1.0ml
Final pH: 6.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 66.15 grams of Part A and 1.0ml of Part B in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Distribute in tubes, bottles or flasks as desired.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

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## Quality Control Specifications

Dehydrated Appearance	Cream to light yellow homogeneous free flowing powder
Prepared Medium	Medium to dark amber coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 6.71% Solution	pH : 6.5 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer (with 5% CO<sub>2</sub>).

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Lactobacillus casei</i> ATCC 9595	50 - 100	good-luxuriant	≥50%
2.	<i>Lactobacillus fermentum</i> ATCC 9338	50 - 100	good-luxuriant	≥50%
3.	<i>Lactobacillus leichmannii</i> ATCC 7830	50 - 100	good-luxuriant	≥50%
4.	<i>Lactobacillus plantarum</i> ATCC 8014	50 - 100	good-luxuriant	≥50%

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

## Test Procedure

- 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.
- Rotate the plate in one direction and then in the reverse direction to distribute the inoculum throughout the medium.
- 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 35-37°C for 3 days, in anaerobic atmosphere supplemented with carbon dioxide.

## Results

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

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

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

## Limitations of the Procedure

- Organisms other than lactobacilli may grow in these media.
- For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
- Consult appropriate texts for detailed information and recommended procedures.

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## Packaging

**Product Name :** Lactobacillus MRS Agar (MRS Agar)

**Product Code :** DM140

**Available Pack sizes :** 100gm/ 500gm

## References

1. Dicks, LMT; M. Silvester; PA Lawson; MD Collins (2000). *International Journal of Systematic and Evolutionary Microbiology* (Society for General Microbiology) 50 (3): 1253-8.
2. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
3. Marshall R.T. (Ed.), 1992, *Standard Methods for the Examination of Dairy Products*, 16th ed., APHA, Washington, D.C.
4. Downes F.P. and Ito K. (Eds.), 2001, *Compendium of Methods for the Microbiological Examination of Foods*, 4th Ed., APHA, Washington, D.C.
5. Sabine and Vaselekos, 1965, *Nature*, 206:960.
6. MacFaddin J., 1985, *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, Vol.1, Williams and Wilkins, Baltimore.

## Further Information

For further information please contact your local MICROMASTER Representative.



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