

MRS Agar Cat. No. MM-M-N043 For the growth of lactobacilli

Principles and uses:

MRS Agar is a selective medium, based on the formulation developed by de Man, Rogosa and Sharpe to provide a medium that would support the good growth of lactobacilli in general, but in particular of those strains which showed poor growth in existing media such as L. brevis and L. fermenti, replacing a variable product (tomato juice).

The medium is suitable for the growth of lactic acid bacteria, including Lactobacillus, Pediococcus and Leuconostoc. Ammonium citrate, at a low pH, inhibits most microorganisms, but allows the growth of Lactobacilli. Dipotassium phosphate and Sodium acetate are buffer agents to maintain a low pH. Tween 80 is an emulsifier. Manganese and Magnesium sulfates are sources of ions and sulfate. Bacteriological peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate. Bacteriological agar is the solidifying agent.

Lactobacilli are microaerophilic and generally require layered plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

Formula per Litre:

Bacteriological agar	10g	Bacteriological peptone	10g	
Dextrose	20g	Dipotassium phosphate	2g	
Magnesium sulfate	0.2g	Manganase sulfate	0.05g	
Beef extract	8g	Sodium acetate	5g	
Tween 80	1g	Yeast extract	4g	
Ammonium citrate	2 g			

Preparation:

Suspend 62 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 12 minutes. Cool to 45-50°C, mix well and dispense into plates.

Instructions for use:

For the enumeration of mesophilic acid lactic bacteria:

- Pour 1 ml of the previously diluted sample into a sterile Petri dish.
- Add a first layer of the cooled medium (45-50°C).
- After solidification, a second layer is poured.
- Incubate the plates up to 3 days at 35°C or up to 5 days at 30°C. If possible, incubate the plates in a CO₂ atmosphere.
- It is important to maintain a humid atmosphere because the plates should not dry out during incubation.
- The growth of some Lactobacillus strains is inhibited at a higher pH of 6.0 and it is necessary to acidify the media to promote growth. To acidify the media some drops of acetic acid can be added.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	6.2 ± 0.2

Technical Data Sheet

Microbiological test:

Incubations conditions: $(30\pm1^{\circ}C / 72\pm3 h)$. Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU)/ Selectivity (10⁴-10⁶ CFU). Reference media: Media batch MRS already validated.

Microorganisms	Specification
Lactobacillus sakei ATCC 15521	Good growth, >70%
Lactococcus lactis ssp. lactis ATCC 19435	Good growth, >70%
Escherichia coli ATCC 25922	Moderate growth

Storage:

Temperature: 2°C - 8°C

Bibliography:

Briggs M (1953) "An Improved Medium for Lactobacilli" J. Dairy Res. 20. 36-40. De Man, J.C. Rogosa, M., Sharpe, Elisabeth (1960) "A Medium for the Cultivation of Lactobacilli". J. Appl. Bact. 23. 130-135

