

## Specification

Solid culture medium for detection, isolation and cultivation of lactobacilli and other lactic acid bacteria from food and beverages according to de Man, Rogosa and Sharpe.

## Presentation

10 Prepared bottle  
Bottle 125 ml  
with: 100 ± 3 ml

### Packaging Details

1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.

### Shelf Life

12 months

### Storage

8-25 °C

## Composition

Composition (g/l):

Peptone proteose.....	10.0
Meat extract.....	8.00
Yeast extract.....	4.00
D(+)-Glucose.....	20.0
Sodium acetate.....	5.00
Triammonium citrate.....	2.00
Magnesium sulfate.....	0.20
Manganese sulfate.....	0.05
Dipotassium phosphate.....	2.00
Polysorbate 80.....	1.00
Agar.....	14.0

## Description /Technique

### Description

MRS Agar is a medium used for the cultivation of lactobacilli. It is a modification of a medium based on the highly nutritious properties of tomato juice. The addition of magnesium, manganese and acetate, together with polysorbate, provides an improved medium for the growth of lactobacilli, including very fastidious species such as *Lactobacillus brevis* and *Lactobacillus fermentum*.

The quality of the peptones in addition to the meat and yeast extracts, combine all the necessary growth factors that make MRS medium one of the best media for the cultivation of lactobacilli.

As the selectivity of this medium is low and contaminants tend to grow subculturing in a (double layer) solid medium, and then in broth is recommended to increase selectivity. In many cases, growth is encouraged by incubation in a CO<sub>2</sub> enriched atmosphere.

MRS medium is particularly recommended for the enumeration and maintenance of lactobacilli either by the MPN technique in broth, or by inoculation on a plate, overlaying it with a second layer of molten medium. This technique overcomes the need for a CO<sub>2</sub> enriched atmosphere.

### Technique:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Spread the plate by streaking methodology or by spiral method. Incubate the plates right side up in a CO<sub>2</sub> atmosphere at 30 ±1°C for 72 ±3h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample , on the specifications,...

This medium can be inoculated directly or after enrichment broth like MRS broth) Incubated under microaerophilic conditions to promote lactobacilli enrichment.

After incubation, enumerate all the colonies that have appeared onto the surface of the agar.

Each laboratory must evaluate the results according to their specifications.

Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by inverse dilution factor if streaked a diluted sample. Report results as Colony Forming Unit (CFU's) per ml or g along with incubation time and temperature.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

**Quality control****Physical/Chemical control**

Color : Yellowish-brown                      pH: 6.2 ± 0.2 at 25°C

**Microbiological control**Melting - pour plates - inoculation Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10<sup>3</sup>-10<sup>4</sup> CFU (qualitative selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosi. Incubation at 30 ±1 °C for 72 ±3 h

Microbiological control according to ISO 11133:2014/A1:2018.

**Microorganism**

*Escherichia coli* ATCC® 25922, WDCM 00013  
*Lactobacillus sakei* ATCC® 15521, WDCM 00015  
*Lactococcus lactis* ATCC® 19435, WDCM 00016  
*Pediococcus pentosaceus* ATCC® 33316, WDCM 00158

**Growth**

Poor to good  
Good (≥70%)  
Good (≥70%)  
Good (≥70%)

**Sterility control**

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

**Bibliography**

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. BocaRaton, Fla. USA
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD, Eds. (2003) Handbook of Culture Media for Food Microbiology. Elsevier Science B.V. Amsterdam
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington DC., USA
- LAWRENCE, D.R. & P.A. LEEDHAM (1979). The detection of acid lactic bacteria. J. Int. Brew. 85:119-121
- ISO Standard 11133:2014 Microbiology of food, animal feed and water. Preparation, production, storage, and performance testing of culture media.
- McFADDIN, J. (1985) Media for the isolation-cultivation-identification-maintenance of medical bacteria. Vol. I. William & Wilkins. Baltimore. USA
- MAN, J.C. de, ROGOSA, M. y SHARPE, M. Elisabeth (1960) A medium for the cultivation of lactobacilli. J. Appl. Bact.; 23:130.
- SMITH, C.E., G.P. CASEY & W.M. INGLEDEW (1987). The use and understanding of media used in Brewing Microbiology. - Update 1987 – Brewer's Digest 62(10)12-16, 43.
- VAN KEER, C., L. van MELKEBEKE, W. VERTRIEST, G. HOOZEE & E. Van SCHOONENBERGHE (1983) Growth of Lactobacillus species on different media. J. Inst. Brew. 89:361-363.