Technical Data Sheet

MRS agar (ISO) Code 84607.0500

Also known as
De Man, Rogosa, Sharpe Agar

Intended use
For the enumeration of lactic acid bacteria from food and animal feeding stuffs (ISO 15214).

Formula * - Composition in g/L

Enzymatic digest of casein	10.00
Meat extract	10.00
Yeast extract	4.00
Glucose	20.00
Dipotassium hydrogen phosphate	2.00
Sodium acetate	5.00
Triammonium citrate	2.00
Magnesium sulphate	0.20
Manganese sulphate	0.05
Agar	13.00
Polyoxyethylenesorbitan monooleate (Tween™ 80)	1.08

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

Final pH 5.7 ± 0.1 at 25 °C.

Instructions for preparation
Dissolve 67.3 g in 1 litre of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes.
If there is a risk of extensive yeast contamination (e.g. in dried sausage), add sorbic acid to the MRS medium as follows.
Dissolve 1,4 g of sorbic acid in about 10 ml of a 1 mol/l solution of sodium hydroxide. Sterilize by filtration. Add this solution to 1 litre of sterilized MRS agar, previously cooled to approximately 47 °C. The final pH of the medium shall be 5.7 ± 0.1 at 25 °C.

Principle of the method and general information
MRS agar, prepared according to the formula of De Man, Rogosa and Sharpe, is designed for the cultivation, enrichment and isolation of *Lactobacillus* spp. from all types of materials. The formula complies with ISO 15214 requirements. Tween® 80, sodium acetate and triammonium citrate intensifies the growth of lactobacilli. Manganese and magnesium are inorganic ions necessary for growth in the presence of citrate.

Instruction for use
For laboratory use only.
- Take two sterile Petri dishes Using a sterile pipette, transfer to each dish 1 ml of the test sample if the product is liquid, or 1 ml of the initial suspension in the case of other products.
- Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution of the test sample if the product is liquid, or 1 ml of the first decimal dilution of the initial suspension in the case of other products.
- Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.

NOTE If high numbers of lactic acid bacteria are expected, it is possible to inoculate only those dilutions necessary to be able to enumerate according to the general case.

- Pour into each Petri dish approximately 15 ml of the MRS agar cooled to approximately 47 °C
- Carefully mix the inoculum with the medium and allow the mixture to solidify.
- Invert the prepared dishes and incubate them at 30 °C for 72 h ± 3 h.
- Count the colonies on the plates containing between 15 and 300 colonies

If lactic acid bacteria other than mesophilic are to be enumerated, incubate the plates at 42 °C for 48 hours (thermophilic lactobacilli) or at 25 °C for 5 days (psicrofilic lactobacilli) or at 30 °C for 48 hours + 22 °C for 48 hours (mesophilic+psicrofilic lactobacilli).
The method described above has been summarized from ISO 15214. Consult the quoted ISO Standard for the details of the procedure for enumeration, results interpretation and confirmation of lactic acid bacteria.

Limitations

- Do not permit the plates to dry out; on drying, acetate concentration increases at surface which inhibits growth of Lactoobacilli.
- As this medium exhibit a poor degree of selectivity, *Pediococcus* and *Leuconostoc* spp. and other secondary bacteria may grow well and compete for nutrients. However, most of these accompanying microorganisms can be inhibited by the addition of various concentrations of selective agents.
- Some *Leuconostoc* spp. may form large slimy colonies which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria.
- Due to the possible development of microorganisms other than lactic acid bacteria on MRS agar, it may be necessary in some cases and for some products to confirm the colonies obtained in by simple techniques (such as Gram staining, or the test for catalase).

Quality Control

Physical characteristics:

Appearance of powder	Yellowish, fine, homogeneous hygroscopic powder
Appearance of prepared medium	Dark yellow, limpid
pH (25°C)	5.7 ± 0.1

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity Ratio
L. sakei ATCC 15521	30 °C / 72 h / AE	QT / 80-120 CFU	Good growth, typical colonies	PR ≥ 0.7
P. pentosaceus ATCC 33316	30 °C / 72 h / AE	QT / 80-120 CFU	Good growth, typical colonies	PR ≥ 0.7
L. lactis ATCC 19435	30 °C / 72 h / AE	QT / 80-120 CFU	Good growth, typical colonies	PR ≥ 0.7
E. coli ATCC 25922	30 °C / 72 h / AE	MM / ≥10^4 CFU	Totally inhibited	
B. cereus ATCC 11778	30 °C / 72 h / AE	MM / ≥10^4 CFU	Totally inhibited	

Notes
PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch
Incubation atmosphere AE: aerobic incubation
Inoculation method QT : quantitative surface plating method; MM: modified Miles-Misra surface drop method
Microbiological characteristics tested in accordance to ISO/TS 11133-2
ATCC is a registered trade mark of American Type Culture Collection

References

- De Man, Rogosa and Sharpe. 1960. J. of App. Bacteriology; 23(1) 130-135.
- ISO 15214:1998 Microbiology of food and animal feeding stuffs — Horizontal method for the emumeration of mesophilic lactic acid bacteria — Colony-count technique at 30 °C

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, at +2 ºC to 8 ºC and <60% RH.

Ordering information

84607.0500	MRS agar (ISO)	Bottle of 500 g

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