TECHNICAL DATA SHEET

MRS AGAR

ENUMERATION OF LACTIC ACID BACTERIA

1 INTENDED USE

MRS Agar is used for the growth and enumeration of cultures of *Lactobacillus* in dairy and other food products as well as in products destined for animal feed.
The medium can be used to culture slow-growing lactobacilli such as *Lactobacillus brevis* and *Lactobacillus fermentum*. Acidified to low pH, it can be used to enumerate *Lactobacillus bulgaricus* in yogurts.
According to the bacteria sought, the media can be adjusted in pH to obtain optimal growth.

The typical composition responds to that described in the standard ISO 15214.

2 HISTORY

De Man, Rogosa and Sharpe developed a formulation in 1960 for a medium specifically designed for the culture of lactobacilli in dairy products, without the need to add tomato juice (an ingredient of highly variable composition).

3 PRINCIPLES

The different peptones, glucose, magnesium and manganese salts supply the nutritive elements required for the growth of lactobacilli.

Tween 80 is composed of a mixture of oleic esters and is a source of fatty acids essential for the growth of these bacteria.

Dipotassium phosphate stabilizes the pH during bacterial growth.

Ammonium citrate and sodium acetate inhibit the development of most contaminants, including streptococci and molds.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media:

- Enzymatic digest of casein ..... 10,00 g
- Meat extract ..... 10,00 g
- Yeast extract ..... 4,00 g
- Glucose ..... 20,00 g
- Tween 80 ..... 1,08 g
- Dipotassium phosphate ..... 2,00 g
- Sodium acetate ..... 5,00 g
- Ammonium citrate ..... 2,00 g
- Magnesium sulfate ..... 0,20 g
- Manganese sulfate ..... 0,05 g
- Bacteriological agar ..... 16,00 g

pH of the ready-to-use media at 25 °C : 5,7 ± 0,1.

5 PREPARATION

Preparation of dehydrated media:

- Dissolve 70,3 g of the dehydrated media (BK089) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring slowly with constant agitation until complete dissolution.
- Dispense into tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain in a molten state at 44-47 °C.

✓ **Reconstitution : 70,3 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of ready-to-melt media:

- Melt the agar for the minimum amount of time in order to obtain complete liquefaction (if it was prepared in advance) or if using the ready-to-melt media (BM089). Do not repeat this operation more than once.
- Cool and maintain in a molten state at 44-47 °C.

NOTES:
According to the protocol used, the agar must be adjusted to the recommended pH before the sterilization cycle.

- For yogurts, it is recommended to use acetic acid in order to reach a final pH of 5,4 ± 0,1.
- Using NaOH, adjust to pH to 6,2 for the enumeration of *Lactobacillus* of *Pediococcus* in products destined for animal feed.
- Using NaOH, adjust to pH 6,5 ± 0.2 for the enumeration of *Bifidobacterium* in products destined for animal feed.

6 INSTRUCTIONS FOR USE

Food microbiology, enumeration of mesophilic lactic acid bacteria (NF ISO 15214)

- Transfer 1 mL of the product to analyze and its serial dilutions to sterile Petri plates.
- Pour in roughly 15 mL of media per plate.
- Homogenize by swirling and let solidify on a cold, flat surface.
- Incubate at 30 ± 1 °C for 72 ± 3 hours in aerobic conditions.

✓ **Inoculation: 1 mL in pour plates**

✓ **Incubation: 72 h at 30 °C**

OU

- On the surface of prepared plates or if using the pre-poured medium (BM239), transfer 0,1mL of the inoculum and its serial dilutions.
- Spread the inoculum on the surface with the aid of a sterile spreader.
- Incubate at 30 ± 1 °C for 72 ± 3 hours in anaerobic or microaerobic conditions.

✓ **Inoculation: 0,1 mL on surface**

✓ **Incubation: Anaerobic ou microaerobic, 72 h à 30 °C**

NOTES:
- For dairy products, incubate under anaerobic conditions at 37°C for 72 hours, refer to ISO 27205, ISO 7889, and ISO 20128 standards.
- For animal feeds for the enumeration of Bifidobacterium (NF EN 15785), inoculate 0,1 mL on the surface and incubate under anaerobic conditions at 37 ± 1°C, for 36 to 48 hours.

7 RESULTS

Enumerate colonies for each plate containing a maximum of 300 colonies. As there is a possibility that other non lactic bacteria may develop, verify under the microscope that the cells are Gram-positive, non-sporulated bacilli.

NOTE:
For the enumeration of Bifidobacterium in animal feeds (NF EN 15785), proceed to count colonies for each plate containing 30 to 300 characteristics colonies.

See ANNEX 1: PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media: cream powder, slightly clumped and brittle.
Prepared media: amber agar.
Typical culture response after 72 ± 3 hours of incubation at 30 ± 1 °C (NF EN ISO 11133)

Microorganisms		Growth (Productivity Ratio: P_R)
Lactobacillus sakei	WDCM 00015	$P_R \geq 70$ %
Lactococcus lactis	WDCM 00016	$P_R \geq 70$ %
Bacillus cereus	WDCM 00001	Inhibited, score 0
Escherichia coli	WDCM 00013	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated media: 2-20 °C.
Ready-to-melt media in vials: 2-8 °C.
Pre-poured media in Petri plates (Ø 90 mm): 2-8°C.
The expiration dates are indicated on the labels.

Prepared media in vials (*): 180 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media:
500 g bottle..... BK089HA

Ready-to-melt media (pH 5,7):
10 x 200 mL vials BM08908

Pre-poured media (pH 5,7):
120 plates (Ø 90 mm) BM23908

11 BIBLIOGRAPHY

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NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media

ISO 27205. February 2010. Norme de composition. Milk products - Acidifying starter cultures - Standard of identity

NF ISO 15214. September 1998. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 degrees Celsius.

ISO 7889. February 2003. Yogurt - Enumeration of characteristic microorganisms - Colony-count technique at 37 °C

ISO 20128. May 2006. Milk products - Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium - Colony-count technique at 37 °C

NF EN 15785. December 2009. Animal feeding stuffs - Isolation and enumeration of *Bifidobacterium* spp.

NF EN 15786. November 2021. Animal feeding stuffs - Isolation and enumeration of *Pediococcus* spp.

NF EN 15787. November 2021. Animal feeding stuffs - Isolation and enumeration of *Lactobacillus* spp.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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ANNEX 1 : PHOTO SUPPORT

MRS Agar

Detection and enumeration of *Lactobacillus*.

Results :

Growth obtained after 72 hours of incubation at 30 °C.

Lactobacillus casei* subsp. *rhamnosus

Characteristic colony:
White color of uniform size.