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# MRS AGAR

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## ENUMERATION OF LACTIC ACID BACTERIA

### 1 INTENDED USE

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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 slowly-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

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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

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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

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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

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### 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  $5.4 \pm 0.1$ .
- 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  $6,5 \pm 0.2$  for the enumeration of *Bifidobacterium* in products destined for animal feed.

## 6 INSTRUCTIONS FOR USE

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### 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  $30 \pm 1$  °C for  $72 \pm 3$  hours in aerobic conditions.

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

✓ **Incubation :**  
72 h at 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.
- In dealing with animal feeds, inoculate on the surface and incubate under anaerobic conditions at  $37 \pm 1$ °C, for 36 to 48 hours for the enumeration of *Bifidobacterium* and 48 to 72 hours for *Lactobacillus* (NF EN 15785; NF EN 15786 ; NF15787).

## 7 RESULTS

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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.

See ANNEX 1 : PHOTO SUPPORT.

## 8 QUALITY CONTROL

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**Dehydrated media :** cream powder, slightly clumped and brittle.

**Prepared media :** amber agar.

Typical culture response after 72 hours of incubation at 30 °C (NF EN ISO 11133)

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

## 9 STORAGE / SHELF LIFE

**Dehydrated media :** 2-20 °C.

**Ready-to-melt media in vials :** 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

## 11 BIBLIOGRAPHY

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Journal Officiel du 4 janvier 1978. Méthode officielle d'analyse pour le dénombrement de la flore spécifique du yaourt ou yoghourt. (arrêté du 25 Novembre 1977).

ISO 27205. Février 2010. Produits laitiers. Ferments acidifiants. Norme de composition.

NF ISO 15214. Septembre 1998. Microbiologie des aliments. Méthode horizontale pour le dénombrement des bactéries lactiques mésophiles. Technique par comptage des colonies à 30 °C.

ISO 7889. Février 2003. Yaourt. Dénombrement des micro-organismes caractéristiques. Technique de comptage des colonies à 37 °C.

ISO 20128. Mai 2006. Produits laitiers. Dénombrement de *Lactobacillus acidophilus* présomptifs sur un milieu sélectif. Technique de comptage des colonies à 37 °C.

NF EN 15785. Décembre 2009. Aliments des animaux. Isolement et dénombrement du *Bifidobacterium* spp.

NF EN 15786. Décembre 2009. Aliments des animaux. Isolement et dénombrement du *Pediococcus* spp.

NF EN 15787. Décembre 2009. Aliments des animaux. Isolement et dénombrement du *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.

Document code : MRS AGAR\_ENV11.

Creation date : 10-2003

Updated : 12-2017

Origin of revision : Update of instructions for use.

## ANNEX 1 : PHOTO SUPPORT

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### 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.

