TECHNICAL DATA SHEET

NON-ANIMAL MRS BROTH

CULTURE OF *LACTOBACILLUS* AND OTHER LACTIC ACID BACTERIA

1 INTENDED USE

The Non-Animal version of MRS broth is used primarily for the growth of lactobacilli or lactic acid bacteria via industrial fermentation for use as starter cultures in varying food, cosmetic or pharmaceutical products. By substituting all animal peptones with non-animal equivalents, the regulatory and safety concerns arising from bovine spongiform encephalothopy and related pathologies can be successfully eliminated, making the medium an ideal fermentative formula providing excellent growth

2 HISTORY

For the culture of lactobacillus, de Man, Rogosa & Sharpe developed in 1960, the composition of a media susceptible to grow lactobacilli used in dairy products without the need to add tomato juice.

More recent concerns centered on BSE/TSE risks in food, cosmetics and pharmaceuticals have led to the substitution in 2003 of meat extract and other animal components by non-animal peptones and/or synthetic ingredients.

3 PRINCIPLES

The different peptones, glucose, manganese and magnesium 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 :

- Peptones (*) ..... 20,00 g
- Yeast extract ..... 5,00 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

pH of the ready-to-use media at 25 °C : 6,0 ± 0,5
(*) : **BK176 is formulated solely with peptones and ingredients of plant (or synthetic) origin**.

5 PREPARATION

- Dissolve 55,3 g of dehydrated media in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution, heating if necessary.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
55,3 g/L

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

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the product to analyze and its serial dilutions to one or several tubes of medium.
- For industrial fermentation, inoculate the broth culture using the appropriate concentration of starter culture for the strain in question.
- Incubate at 30°C or at 37°C, from 48 hours to 5 days according to the microorganisms being studied or the pertinent industrial production protocol.

✓ **Inoculation :**
1 mL

✓ **Incubation :**
48 h to 5 days at 30 or 37 °C

7 RESULTS

Examine the tubes containing characteristic cloudiness of microbial growth. In addition to lactobacilli, *Leuconostoc* and *Pediococcus* may also develop. It is recommended to prepare subcultures on an appropriate media. Depending on the qualitative results obtained, use the most probable number method for enumeration.

Growth in a fermentative context will depend on strain, conditions, material and the specific protocols used for harvesting biomass or yield-dependent criteria.

8 QUALITY CONTROL

Dehydrated media : cream powder, slightly clumped.
Prepared media : amber solution, may contain a slight precipitate.

Typical culture response after 48 hours of incubation at 37 °C, inoculum ≤ 100 microorganisms

Microorganisms		Growth
Lactobacillus delbrueckii subsp. *lactis*	ATCC® 4797	Positive
Lactobacillus casei subsp. *rhamnosus*	WDCM 00101	Positive
Lactobacillus plantarum	ATCC 8014	Positive
Lactobacillus fermentum	ATCC 9338	Positive

9 STORAGE / SHELF LIFE

Dehydrated media : 2-20 °C.
The expiration date is indicated on the label..
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Prepared media in tubes or 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 BK176HA
Other industrial formats may be available ..... Inquire

11 BIBLIOGRAPHY

de Man, J.C., Rogosa, M., and Sharpe, M.E.. 1960. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology, **23** : 130-135.

MacFaddin, J.F.. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilklins, Baltimore, volume 1: 543-545.

ISO 9232 / IDF 146. Février 2003. Yaourt. Identification des micro-organismes caractéristiques (*Lactobacillus delbrueckii* subsp. *bulgaricus* et *Streptococcus thermophilus*).

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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