



MRS BROTH WITH TWEEN 80

Dehydrated culture medium

1 - INTENDED USE

For the cultivation of *Lactobacillus* spp. and other lactic acid bacteria in dairy products and other foods as well as in products intended for animal feed.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.00 g
Beef extract	10.00 g
Yeast extract	5.00 g
Glucose	20.00 g
Dipotassium hydrogen phosphate	2.00 g
Sodium acetate	5.00 g
Ammonium citrate	2.00 g
Magnesium sulphate	0.20 g
Manganous sulphate	0.05 g
Tween® 80	1.00 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactobacilli are lactic acid bacteria, a group that also includes, among others, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*. All these species can produce lactic acid in considerable amounts. They are Gram-positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by microaerobic conditions.

MRS Broth with Tween 80 is based on the formulation devised by de Man, Rogosa and Sharpe¹ in 1960, primarily for the cultivation of lactobacilli from oral, faecal, dairy and other sources, with the intention of producing a defined medium as a substitute of tomato juice.

The medium allows a luxuriant growth of all strains of lactobacilli, and more particularly strains with slow and difficult development such as *L. brevis* and *L. fermenti*. MRS Broth with Tween 80 is slightly selective for lactobacilli and some growth of leuconostocs and pediococci may occur. Selectivity can be improved by the addition of selective compounds such as sorbic acid, adapting the incubation temperature and decreasing the pH: lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range.^{2,3} MRS Broth is recommended by APHA⁴ to screen heterofermentative lactic acid producers with MPN procedure.

Essential growth factors are provided by peptones and yeast extract which are sources of nitrogen, carbon, vitamins and minerals. Polysorbate 80 provides the fatty acids necessary for the metabolism of lactobacilli while magnesium sulphate and manganese sulphate provide essential ions for the multiplication of lactobacilli. Glucose is the fermentable carbohydrate and a source of carbon and energy for microbial growth. Dipotassium phosphate buffers the medium. Selectivity is provided by the presence of ammonium citrate and sodium acetate which, at low pH, allows the growth of lactic acid bacteria while inhibiting a number of other groups of microorganisms.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 55.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into tubes or bottles of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	brown, limpid
Final pH at 20-25 °C	6.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
MRS Broth with Tween® 80	Dehydrated medium	4017292	500 g (9.1 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

8 - SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

The sample and its dilutions can be inoculated directly into MRS Broth with Tween 80. Choose incubation time, temperature and atmosphere based on the research to be performed (e.g at 35°C for 4 days, or at 30°C for 5 days, in an aerobic atmosphere).

APHA⁴ describes a MPN procedure for detecting lactic acid producers: MRS Broth tubes containing inverted Durham tubes are inoculated in a three-tube MPN method; after incubation at 35°C for 4 days, tubes showing gas formation are counted as presumptive for heterofermentative organisms.

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.





CONTROL STRAINS	INCUBATION T° / T - ATM	EXPECTED RESULTS
<i>L. sakei</i> ATCC 15521	30-37° / 72 H-A	growth
<i>P. damnosus</i> ATCC 29358	30-37° / 72 H-A	growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated MRS Broth with Tween 80 is tested for productivity by comparing the results with a previously approved Reference Batch. Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 72 hours and recording the highest dilution showing growth in Reference Batch ($G_{R_{RB}}$) and in Test Batch ($G_{R_{TB}}$). Productivity is tested with the target strains *L. acidophilus* ATCC 314, *L. delbrueckii* subsp. *bulgaricus* DSM 20081, *L. sakei* ATCC 15521, *L. fermentum* ATCC 9338, *L. lactis* ATCC 11454. The productivity index ($G_{R_{RB}}-G_{R_{TB}}$) for each test strain shall be ≤ 1 .

13 – LIMITATIONS OF THE METHOD

- L. mesenteroides* and *L. dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, and can grow on MRS Broth. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine.²
- If there is a risk of extensive yeast contamination (e.g., in dried sausage), add sorbic acid to the medium.²

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).










The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 – REFERENCES

- DeMan JC, Rogosa M, Sharpe ME. (1960). An improved medium for the cultivation of Lactobacilli. 1960; J Appl Bact 23,130-135.
- ISO 15214:1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30°C.
- Reuter G. Elective and selective media for lactic acid bacteria. Int J Food Microbiol 1985; 2:55-68.
- Njongmenta NA et al. APHA Compendium of Methods for the Microbiological Examination of Foods. Chapter 19 Acid-producing microorganism. American Public Health Association, Washington D.C. 5th Ed, 2015

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TABLE OF APPLICABLE SYMBOLS

 or REF Catalogue number	 Batch code	 Manufacturer	 Store in a dry place	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

