MRS AGAR WITH TWEEN® 80

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

For the detection and enumeration of *Lactobacillus* and other lactic acid bacteria in dairy products and other food products as well as in products intended for animal feed.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED AND READY-TO-USE PLATES

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
Diammonium citrate	2.00 g
Magnesium sulphate	0.20 g
Manganous sulphate	0.05 g
Agar	15.00 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 Agar with Tween 80 is based on the formulation devised by Johannes Cornelis de Man, Morrison Rogosa and Margaret Elisabeth Sharpe[1] 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 agar.

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 Agar 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 or antibiotics, 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]

Peptones provide nitrogen and minerals for microbial growth; yeast extract is a source of B-vitamins complex for growth stimulation. Tween® 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.

MRS Agar with Tween 80 differs from MRS Agar ISO Formulation (REF 401728S) in that it contains ammonium citrate bibasic instead of tri-ammonium citrate and in the final pH.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 70.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise in the autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
MRS Agar with Tween® 80	Dehydrated medium	4017282	500 g (7.1 L)
MRS Agar with Tween® 80	Ready-to-use plates	541728	2 x 10 plates ø 90 mm

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

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of MRS Agar with Tween 80, cooled to approximately 47°C into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.

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Choose incubation time, temperature and atmosphere based on the research to be performed (e.g 35°C for 3 days, or 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide). Consult the cited references for further information.[5,6]
MRS Agar with Tween 80, acidified at pH 5.4 ± 0.1 with acetic acid, may be used for the enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* in yogurt according to the method described by ISO 7889:[4] transfer with a sterile pipette 1 ml of each dilution into Petri dishes and pour 15 ml of acidified MRS Agar. Mix the inoculum with the medium, allow the mixture to solidify and incubate in anaerobic conditions at 37 °C for 72 h.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Lactobacilli grow with lenticular, often sharp-shaped, colonies of diameter 1 mm to 3 mmm, embedded in or on MRS Agar.
Count the colonies on plates having between 15 and 300 colonies.

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
L. sakei ATCC 15521	30°/ 72 H-A	growth
E. coli ATCC 25922	30°/ 72 H-A	inhibited

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 and ready-to-use MRS Agar with Tween 80 (TB:Test Batch) is assessed for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative method with the target strains *L. sakei* ATCC 15521, *P. pentosaceus* ATCC 33316, *L. lactis* ATCC 19435. The plates are inoculated by pour plate technique with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 72 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU_{TB}/CFU_{RB})) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and size are typical the results are considered acceptable and conform to the specifications.
The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *E. coli* ATCC 25922 and *B. cereus* ATCC 11778. The growth of non-target strains is inhibited.

13 – LIMITATIONS OF THE METHOD

- 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.
- *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, and can grow on MRS Agar. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine.
- Due to the possible development of microorganisms other than lactic acid bacteria, it may be necessary in some cases and for some products to confirm the colonies by simple techniques (such as Gram staining, or the test for catalase).[2]
- If there is a risk of extensive yeast contamination (e.g., in dried sausage), add sorbic acid to the medium.[2]
- Do not permit plates to dry out; on drying, acetate concentration increases at surface which inhibits growth of lactobacilli.[7]

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.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- 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 Sheets 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

Ready to use plates

Upon receipt, store plates in their original pack at +2°C / +8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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 (plates/flasks) and the applied storage conditions (temperature and packaging). According to Baird RM et al. the prepared plates may be stored at 2-8°C for 14 days.[8]

16 – REFERENCES

1. DeMan JC, Rogosa M, Sharpe ME. (1960). An improved medium for the cultivation of Lactobacilli. 1960; J Appl Bact 23,130-135.
2. 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.
3. Reuter G. Elective and selective media for lactic acid bacteria. Int J Food Microbiol 1985; 2:55-68
4. ISO 7889 (IDF 117): 2003. Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C.
5. 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
6. Schoeni JL. APHA Compendium of Methods for the Microbiological Examination of Foods. Chapter 20 Probiotics. American Public Health Association, Washington D.C. 5th Ed, 2015
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:228-232.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2023/01

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

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