M17 AGAR

Dehydrated culture medium

1 - INTENDED USE

For the cultivation and enumeration of lactic streptococci in milk and dairy products; for the enumeration of *Streptococcus thermophilus* in yogurt.

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

Tryptic digest of casein	2.50 g
Peptone	2.50 g
Soy peptone	5.00 g
Yeast extract	2.50 g
Beef extract	5.00 g
Sodium glycerophosphate	19.00 g
Magnesium sulphate	0.25 g
Ascorbic acid	0.50 g
Lactose	5.00 g
Agar	13.00 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactic streptococci are acid-producing bacteria, nutritionally fastidious, requiring complex media for optimum growth. They are used extensively as starter cultures in the dairy industry.

M17 Agar was developed by Terzaghi and Sandine[1] for the improved growth of lactic streptococci and their bacteriophages by adding disodium-β-glycerophosphate as a buffer system to the medium M16 devised by Lowrie RJ et al.[2] It was later found that the addition of glycerophosphate buffer inhibits the growth of many *Lactobacillus* species.[3]

M17 Agar is recommended by ISO 7889 (IDF 117) for the enumeration of *Streptococcus thermophilus* from yogurt.[4] Terzaghi and Sandine recommend M17 Agar also for the demonstration of lactic bacteriophage activity; when this method is adopted, 100mL of medium is supplemented with 10 mL $CaCl.6H_2O$ 1.0 M.[1] The medium also proved useful for isolation of bacterial mutants lacking the ability to ferment lactose; such mutants formed minute colonies on M17 agar plates, whereas wild-type cells formed colonies 3 to 4 mm in diameter.[1]

The plant protein extract (soy peptone) and other peptones provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is the fermentable carbohydrate and a source of carbon and energy. Sodium glycerophosphate buffers the acidity produced by lactose fermentation and maintains the pH above 5.7 during the active microbial growth, allowing the optimal recovery of lactic streptococci and the inhibition of many lactobacilli. Ascorbic acid stimulates growth of lactic streptococci while magnesium sulphate provides essential ions for growth. The calcium-containing medium is used for the assay of bacteriophages of lactic streptococci.[1]

As indicated by the Pharmacopoeia of Culture Media for Food Microbiology[5] lactose is included in the formulation, however Biolife makes lactose-free medium available (M17 Agar w/o Lactose REF 401719S2).

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 and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
M17 Agar	Dehydrated medium	4017192	500 g (9.1 L)

The medium is also available without lactose at the customer's request: M17 Agar w/o Lactose REF 401719S2, 500 g.

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

Milk and dairy products. 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

For the enumeration of *S.thermophilus* in yogurt, ISO 7889[4] recommends the following technique:

1. Accurately mix the contents of the yoghurt pot by using a sterile spatula. In the case of fruit-yogurt homogenise the contents of the pot for one minute.
2. Weigh 10 g of product in a 200 mL bottle and bring to 50 g with an autoclaved diluent as specified in ISO 6887-1[6] and ISO 6887-5[7](e.g., 0.1% peptone solution).
3. Blend for 1 min with the blender. Dilute to 100 g with the diluent to obtain a 10^{-1} dilution.
4. Prepare a suitable series of decimal dilutions of the sample suspension in 9 mL of diluent.
5. From each tube, pipette 1 mL of the appropriate dilution in a 90 or 100mm Petri dish in duplicate.
6. Pour 15 mL of M 17 Agar, cooled to 45°C to each dish. Mix the inoculum with the medium and allow the mixture to solidify.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

7. Incubate at 37°C for 48 hours.

10 - READING AND INTERPRETATION

After incubation, examine the plates under subdued light. Count the colonies on plates containing between 15 and 300 colonies.
S. thermophilus forms lenticular colonies of diameter 1 mm to 2 mm. Under a microscope, these microorganisms appear as spherical or ovoid cells (of diameter 0.7 µm to 0.9 µm) in pairs or in long chains. They are Gram-positive and catalase-negative.
Take care not to mistake particles of undissolved sample or precipitated matter for pinpoint 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
S. thermophilus ATCC 19258	37°C / 48 hours /A	good 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 M17 Agar is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
The productivity is tested by a quantitative method with the target strains *S. thermophilus* ATCC 19258 and *S. lactis* ATCC 11454 and the enumeration of *S. thermophilus* in a yogurt sample. The poured plates are inoculated with decimal dilutions in saline of a colonies' suspension or of the yogurt dilutions and incubated at 37°C for 48 hours. The colonies are enumerated on both batches and the productivity ratio (Pr) 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 by a quantitative poured plate method with the non-target strains: *L. acidophilus* ATCC 314 and *L. delbrueckii* subsp. *bulgaricus* DSM 20081 The growth of non-target strains is partially inhibited and the growth exhibits small pinpoint colonies.

13 – LIMITATIONS OF THE METHOD

- Some strains of *L. delbrueckii* subsp. *bulgaricus* may form small pinpoint colonies on the M17 Agar, especially with samples of yogurt presenting a much higher number of lactobacilli compared to the number of streptococci.[4]

14 - PRECAUTIONS AND WARNINGS

- The 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.
- 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 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 +10°C /+30°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). According to Curtis GDV and Baird RM,[5] the self-prepared medium may be stored at +2°C/+8°C for 7 days.

16 – REFERENCES

1. Terzaghi BE, Sandine WE. Improved medium for lactic streptococci and their bacteriophages. App Microbiol 1975;29 (6): 807–813.
2. Lowrie RJ, Pearce LE. The plating efficiency of bacteriophages of lactic streptococci. NZ J Dairy Sci Technol 1971; 6: 166–171.
3. Shankar PA, Davies FL. A note on the suppression of Lactobacillus bulgaricus in media containing β-glycerophosphate and application of the media to selective isolation of Streptococcus thermophilus from yoghurt. Int J Dairy Technol 1977;30 (1):28–30.
4. ISO 7889 (IDF 117): 2003. Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C.
5. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:214-15.
6. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

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E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

7. ISO 6887-5:2020 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5: Specific rules for the preparation of milk and milk products.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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.

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