

M17 BROTH

Dehydrated culture medium

1 - INTENDED USE

For the cultivation of lactic streptococci in milk and dairy products.

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

*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 Broth is based on the formulation developed by Terzaghi and Sandine¹ 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 Broth use in conjunction with M17 Agar in bacteriophage assays has been described by Terzaghi and Sandine¹ who also suggested that M17 Broth would be a suitable medium for the maintenance of starter cultures.

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.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 42.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute and sterilise in the autoclave at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellowish, limpid
Final pH at 20-25 °C	7.1 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
M17 Broth	Dehydrated medium	4017202	500 g (11.9 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

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

Inoculate the tubes and incubate 24-48 hours at 37 °C (or other suitable temperature) aerobically.

10 - READING AND INTERPRETATION

After incubation, the streptococcal growth is evidenced by turbidity in the broth.

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>S. thermophilus</i> ATCC 19258	37°C / 48 ore /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 Broth is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.





The 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 48 hours and recording the highest dilution showing growth in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the target strains *S. thermophilus* ATCC 19258, *S. lactis* ATCC 11454 and with a yogurt sample containing *S. thermophilus*. The productivity index $G_{RB}-G_{TB}$ for each test strain shall be ≤ 1 . The selectivity is evaluated with by dilution to extinction 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.

13 – LIMITATIONS OF THE METHOD

- Some strains of *L. delbrueckii* subsp. *bulgaricus* may exhibit a slight growth on M17 Broth.

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).

16 – REFERENCES

- Terzaghi BE, Sandine WE. Improved medium for lactic streptococci and their bacteriophages. *App. Microbiol* 1975;29 (6): 807–813.
- Lowrie RJ, Pearce LE. The plating efficiency of bacteriophages of lactic streptococci. *N.Z. J. Dairy Sci. Technol* 1971; 6: 166–171.
- 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.

TABLE OF APPLICABLE SYMBOLS

 or  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 1	Updated layout and content	2022/08

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

