

# LISTERIA ENRICHMENT BROTH

## Dehydrated culture medium

### 1 - INTENDED USE

Selective enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food and environmental samples.

### 2 - COMPOSITION - TYPICAL FORMULA \*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptic digest of casein	17.0 g
Soy peptone	3.0 g
Yeast extract	6.0 g
Sodium chloride	5.0 g
Glucose	2.5 g
Dipotassium hydrogen phosphate	2.5 g
Acriflavine HCl	15.0 mg
Nalidixic acid	40.0 mg
Cycloheximide	50.0 mg

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

### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Listeria Enrichment Broth (LEB) is based on the formula devised by Lovett et al. in 1987 as an alternative to the cold enrichment procedure.<sup>1</sup> This medium has been shown to recover an inoculum of less than 10 CFU/mL from raw milk.

Casein peptone and soy peptone provide essential nitrogen, carbon-based nutrients, and trace elements for microbial growth; yeast extract is a source of vitamins, particularly of the B-group, for growth stimulation. Glucose is a carbohydrate that increases the growth rate of *Listeria*; dipotassium phosphate is used as buffering agent to control the pH in the medium; sodium chloride maintains osmotic balance. Selectivity is provided by cycloheximide, an antifungal compound, nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity. Because all these antimicrobials are thermostable, they are included in the powdered medium and can be sterilised by autoclaving.<sup>2,3</sup>

### 4 - DIRECTIONS FOR MEDIA PREPARATION

Suspend 36.1 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH of complete media (at 20-25°C)	7.3 ± 0.2

### 6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Listeria Enrichment Broth	Dehydrated medium	4016012	500 g (13.8 L)
		4016014	5 kg (138.5)

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

### 8 - SPECIMENS

Foods, dairy products, environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards

### 9 - TEST PROCEDURE

- Add a test portion of 25 g or 25 mL to 225 mL of Listeria Enrichment Broth and homogenize.
- Incubate at 30 °C for 48 h.
- Streak the enrichment broth onto one or more plating-out media (e.g., ALOA, Oxford or PALCAM plates).
- Incubate the agar plates at 37°C for 24-24 ± 2 hours.

### 10 - READING AND INTERPRETATION

After incubation, *Listeria* spp. produce a turbidity into the enrichment broth.

After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Follow the procedure described by International Standards for the identification of 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.<sup>2</sup>

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>L. monocytogenes</i> ATCC 19111	30°C / 48h / A	growth
<i>S. aureus</i> ATCC 25923	30°C / 48h / 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 *Listeria* Enrichment Broth is tested for productivity and selectivity 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 30°C for 48 hours and recording the highest dilution showing growth in Reference Batch (Gr<sub>RB</sub>) and in Test Batch (Gr<sub>TB</sub>). Productivity is tested with the following target strains: *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 13932. The productivity index Gr<sub>RB</sub>-Gr<sub>TB</sub> for each test strain shall be ≤ 1.

Productivity and selectivity are tested together with mixtures of approximately 100 CFU of target organisms and 1000 CFU of non-target organisms per test tubes, incubating at 30°C for 48 hours. Mixture of target and non-target strains: *L. monocytogenes* ATCC 19111+*E. coli* ATCC 25922+*E. faecalis* ATCC 29212. After incubation of inoculated tubes and sub-culture on ALOA plates, the target strains will show more than 10 colonies per plate.

Moreover, selectivity is tested by inoculating approximately 1000 CFU per tube of the following non-target strains: *E. faecalis* ATCC 29212, and *E. coli* ATCC 25922. After incubation *E. faecalis* exhibits a growth with less than 100 UFC after subculture on Tryptic Soy Agar while *E. coli* is totally inhibited. Selectivity is tested also with the non-target strain *C. albicans* ATCC 18804 by dilution to extinction method: the strain is totally inhibited.

### 13 - LIMITATIONS OF THE METHOD

- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *L. monocytogenes* must be confirmed by suitable tests.
- Injured *Listeria* cells may fail to grow on this medium.<sup>4</sup>
- Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or national regulations for the complete procedures.

### 14 - PRECAUTIONS AND WARNINGS

- This product 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.
- *Listeria* Enrichment Broth is classified as hazardous. 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](http://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 culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized tubes 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 product are available on the website [www.biolifeitaliana.it](http://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



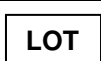







Upon receipt, store at +10/+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, presence of 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

1. Lovett J, Francis DW, Hunt JM. *Listeria monocytogenes* in Raw Milk: Detection, Incidence, and Pathogenicity. *J Food Prot* 1987; 50:188-19
2. Martindale The Extra Pharmacopoeia (1982) Twenty-eighth Edition. The Pharmaceutical Press, London.
3. Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Practical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.
4. APHA Compendium of Methods for the Microbiological Examination of foods 4<sup>th</sup> ed.

### 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	2022/07
Revision 2	Autoclaving temperature	2025/05

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

