



LAURYL PEPTO BIOS BROTH (Lauryl Sulphate Broth-Lauryl Tryptose Broth) Dehydrated culture medium

1 - INTENDED USE

Selective medium for detection and enumeration of *Escherichia coli*, coliforms and faecal coliforms in materials of sanitary importance.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of plant and animal tissues	20.00 g
Lactose	5.00 g
Sodium chloride	5.00 g
Sodium lauryl sulphate	0.10 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 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

Lauryl Pepto Bios Broth, also known as Lauryl Sulfate Broth or Lauryl Tryptose Broth or Lauryl Sulfate Tryptose Broth, is prepared according to the formulation devised by Mollan and Ourby.¹

This medium is recommended by ISO 7251² for detection and enumeration of *E. coli* in foodstuffs, by ISO 4831³ for the detection of coliforms in foodstuffs, by FDA-BAM⁴ for the MPN test for coliforms, faecal coliforms and *E. coli* in foods, bottled water, seawater and shellfish, by APHA⁵⁻⁷ for the detection of coliforms in water, dairy products and other foods.

The broth is specifically designed to allow rapid multiplication and copious gas production from a small inoculum of target organisms.⁸ Essential growth factors are provided by enzymatic digest of plant and animal tissues which is a source of nitrogen, carbon, amino acids and minerals; lactose is a fermentable carbohydrate. Phosphates are used as buffering agents to control the pH in the medium and sodium chloride maintains the osmotic balance. The surface-active agent sodium lauryl sulphate acts as the selective agent in restricting the growth of bacteria other than coliforms.⁸

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.6 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into 16 x160 mm test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength suspend 71.2 g in 1000 mL of cold purified water and dispense 10 mL into 20x200 mm tubes. The Durham tubes shall not contain air bubbles after sterilization.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lauryl Pepto Bios Broth	Dehydrated medium	4015802	500 g (14 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.^{2,3} Foods, bottled water, seawater and shellfish.⁴ Water, dairy products and other foods.⁵⁻⁷ For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Test for detection and enumeration of presumptive *E. coli*.²

- Inoculate tubes of Lauryl Pepto Bios Broth at single and double strength.
- For enumeration, follow the MPN scheme with 3 single-strength and 3 double-strength tubes inoculated with the sample suspension and its decimal dilutions. For detection method inoculate one tube with single strength medium or one tube with the double strength medium with the initial suspension, depending of the limit required.
- Incubate at 37 °C ± 1 for 24 h ± 2 h. If, at this stage, neither gas production nor opacity preventing the observation of gas production is observed, incubate for up to 48 h ± 2 h
- From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth showing opacity, cloudiness or any visible gas, inoculate with a sampling loop a tube of EC Broth (REF 401425).
- Incubate the EC Broth tubes at 44 °C ± 1°C for 24 h ± 2 h. If, at this stage, there is no visible gas in the EC Broth, extend the incubation up to a total of 48 h ± 2 h.
- For the confirmatory test of *E. coli* proceed as following:
- After incubation, if visible gas is observed, inoculate a tube of Peptone (Tryptone) Water (REF 401891), preheated to 44 °C, using a sampling loop.
- Incubate for 48 h ± 2 h at 44 °C.
- Add 0.5 mL of Kovacs' Reagent (REF 19171000) to the incubated tubes, mix well and examine after 1 min. A red colour in the alcoholic phase indicates the presence of indole.



**Test for detection and enumeration of presumptive coliforms.³**

1. Inoculate tubes of Lauryl Pepto Bios Broth at single and double strength.
2. For enumeration, follow the MPN scheme with 3 single-strength and 3 double-strength tubes inoculated with the sample suspension and its decimal dilutions. For detection method inoculate one tube with single strength medium or one tube with the double strength medium with the initial suspension, depending of the limit required.
3. Incubate the tube of double-strength medium at 30 °C or 37 °C (as agreed) for 24 h ± 2 h.
4. Incubate the tube of single-strength medium at 30 °C or 37 °C (as agreed) for 24 h ± 2 h or, if neither gas formation nor opacity prevent this, gas formation is observed at this stage, continue incubation for another 24 h ± 2 h.
5. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth inoculate with a loop a tube of Brilliant Green Bile Broth 2% (REF 401265) and incubate at 30 °C ± 1 °C or 37 °C ± 1 °C for 24 h ± 2 h or, if gas formation is not observed, extend the incubation up to a total of 48 h ± 2 h.

10 - READING AND INTERPRETATION

Lauryl Pepto Bios Broth becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.

Consider as positive for *E. coli* bacteria the growth in Lauryl Pepto Bios Broth that has given rise to visible gas in tube of EC Broth and to indole production.²

Consider as coliforms the growth in Lauryl Pepto Bios Broth that show gas formation in Brilliant Green Bile Broth tube.³

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>E. coli</i> ATCC 25922	37°C/24-48 H/A	growth, with gas production
<i>C. freundii</i> ATCC 43864	37°C/24-48 H/A	growth, with gas production
<i>E. faecalis</i> ATCC 19433	37°C/24-48 H/A	growth partially 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 Lauryl Pepto Bios Broth is tested for productivity, specificity 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 37°C and 30°C for 24 and 48 hours and recording the highest dilution showing growth and gas production in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the following target strains: *E. coli* ATCC 25925, *E. coli* ATCC 8739, *C. freundii* ATCC 43864, *K. pneumoniae* ATCC 27736. The productivity index $G_{RB} - G_{TB}$ for each test strain shall be ≤ 1 and the tubes shall exhibit gas. Specificity is tested with appropriate dilutions of non-target strain *S. Typhimurium* ATCC 14028. After incubation, the strain exhibits good growth without gas production.

Selectivity is tested with appropriate dilutions of non-target strain *E. faecalis* ATCC 19433. After incubation, the growth of the non-target strain is partially inhibited.

13 - 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.
- 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 the 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 culture medium 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 product 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.

14 - 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 Baird RM *et al.*, the prepared Lauryl Pepto Bios Broth can be stored in the refrigerator for up to 1 month in screw-capped containers.⁸

15 - REFERENCES

1. Mallmann WL, Darby CW. Uses of a Lauryl Sulfate Tryptose Broth for the Detection of Coliform Organisms. *Am J Public Health Nations Health*.1941 Feb;31(2):127-34.
2. ISO 7251:2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique,
3. ISO 4831:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique
4. FDA-BAM Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. Content current as of:10/09/2020
5. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.
6. APHA Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., 2015.
7. APHA Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., 2004.
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; 195-196.

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 1	Updated layout and content	2022/07

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

