TRYPTIC SOY YEAST EXTRACT BROTH (TSYEB)

Dehydrated culture medium

1 - INTENDED USE

General purpose medium for the cultivation of a wide variety of microorganisms, especially *Listeria* spp.

2 – COMPOSITIONS

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

Enzymatic digest of casein	17.0 g
Enzymatic digest of soya meal	3.0 g
Yeast extract	6.0 g
Sodium chloride	5.0 g
Dipotassiurn hydrogen phosphate	2.5 g
Glucose	2.5 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

Tryptic Soy Yeast Extract Broth is based on the formulation of Tryptic Soy Broth to which yeast extract is added. It is a general-purpose medium for the cultivation of a wide variety of microorganisms and it is recommended by FDA-BAM[1] and APHA[2] for the cultivation of suspected *Listeria* spp. colonies isolated on selective media.
Casein and soy peptones and yeast extract provide nitrogen, carbon, amino acids, vitamins and minerals required for the microbial growth. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium while sodium chloride maintains the osmotic equilibrium.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 36 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute into tubes and sterilise by autoclaving at 121ºC for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, clear
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Soy Yeast Extract Broth	Dehydrated medium	4021672	500 g (13.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Colonies cultivated on selective plating media.

9 - TEST PROCEDURE

Pick typical colony for inoculate a tube of TSYEB. Incubate at 35°C for 24 h. This culture may be kept at 4°C several days and used repeatedly as inoculum.[1]
Use this culture for carbohydrate fermentation test and motility test.[1,2]
Carbohydrate fermentation test: from TSYEB culture, inoculate the following carbohydrates in 0.5% solutions in Fermentation Broth Base (REF 401488) with Durham tubes: dextrose, esculin, maltose, rhamnose, mannitol, and xylose. Incubate 7 days at 35°C.[1]
Motility test: from TSYEB culture, inoculate a tube of SIM Bios Medium (REF 402036); observe for an umbrella-like growth pattern during 7 days of incubation at ambient temperature.[2]

10 - READING AND INTERPRETATION

The presence of microorganisms in TSYEB is indicated by a varying degree of turbidity, specks and flocculation in the medium.

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. monocytogenes NCTC 7973	35 or 25°C/ 24 H/A	good growth
L. monocytogenes ATCC 13932	35 or 25°C/ 24 H/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated TSYEB (Test Batch:TB) is tested for productivity by comparing the results with a Reference Batch (RB).
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 24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).

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Productivity is tested with the following target strains: *L. monocytogenes* ATCC 19111 and *L. innocua* ATCC 33090. Productivity index (Gr_{RB}-Gr_{TB}) for each test strain shall be ≤ 1.
Moreover, productivity is tested according to ISO 11133 method by incubating at 25°C for 24 hours with the following strains: *L. monocytogenes* NCTC 7973, *L. monocytogenes* ATCC 13932. Both target strains exhibit a good growth.

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 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.
- 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).

15 – REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. Rev April 2022.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

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	2022/10

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

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