



TRYPTIC SOY YEAST EXTRACT AGAR (TSYEA)

Dehydrated and ready-to-use 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
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Agar	12.0 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 Agar (TSYEA) is based on the formulation of Tryptic Soy Broth to which yeast extract and agar are added. It is a general-purpose medium for the cultivation of a wide variety of microorganisms and it is recommended by ISO 11290^{1,2} for the purification of the colonies cultivated on selective isolation 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 48 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates 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 Agar (TSYEA)	Dehydrated medium	4021662	500 g (10.4 L)
Tryptic Soy Yeast Extract Agar (TSYEA)	Ready-to-use plates	542166	2 x 10 plates ø 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 – SPECIMENS

Colonies cultivated on selective plating media

9 - TEST PROCEDURE

Streak the selected colonies onto the surface of pre-dried plates of TSYEA, to allow the isolated colonies to develop.

Incubate at 37 °C for 18 h to 24 h or until growth is satisfactory.

The obtained colonies will then be used for biochemical identification tests.

10 - READING AND INTERPRETATION

Typical colonies of *Listeria* spp. on Tryptic Soy Yeast Extract Agar are 1 mm to 2 mm in diameter, convex, colourless and opaque with an entire edge. When the plates are held to the light (artificial or natural) at about 45-degree angle, colonies exhibit a blue-grey colour and a granular surface.

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>L. monocytogenes</i> NCTC 7973	37°C/ 24 H/A	good growth
<i>L. monocytogenes</i> ATCC 13932	37°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 TSYEA (Test Batch:TB) is tested for productivity by comparing the results with a Reference Batch (RB).

Productivity is tested by quantitative method with the target strains *L. monocytogenes* NCTC 7973 and *L. monocytogenes* ATCC 13932. The plates are inoculated by surface spreading technique with decimal dilutions in saline of a colonies' suspension and incubated at 37 °C





for 24 hours in air. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU_{TB}/CFU_{RB}) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications.

13 - PRECAUTIONS AND WARNINGS

- This culture 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.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- 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 Sheets of the products 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

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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 (plates/flasks) and the applied storage conditions (temperature and packaging).

15 – REFERENCES

- ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method.
- ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 2: Enumeration method.

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	single-use

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.

