

INSTRUCTIONS FOR USE

NUTRIENT AGAR

Dehydrated culture medium


Salmonella Typhimurium on Nutrient Agar

1 - INTENDED USE

In vitro diagnostic. General purpose medium for the cultivation, subculture and purification of colonies of non-fastidious microorganisms, isolated from clinical and non-clinical specimens.

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

Beef extract	3 g
Peptone	5 g
Agar	15 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

Nutrient Agar is a culture medium based on meat peptones, used for the cultivation of non-fastidious microorganisms. The peptones provide carbon, nitrogen and vitamins for the growth of most non-fastidious microorganisms (e.g., enterobacteria, staphylococci). The absence of sodium chloride limits the swarming of *Proteus* spp. The formulation of the Nutrient Agar complies with the recommendations of ISO 6579¹ and ISO 10273². Nutrient Agar was one of the first media utilised in microbiology and can still be used for the examination of water and food for preparing stock cultures, for the preliminary cultivation of a sample undergoing successive bacteriological examinations, and for the isolation of microorganisms in pure culture.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Agar	Dehydrated medium	4018102	500 g (21.7 L)
		4018104	5 kg (217 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator, laboratory equipment as required, sterile loops, swabs, Petri dishes, tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Generally Nutrient Agar is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of clinical specimens.

9- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Routinely, incubate at 35-37°C in aerobic conditions for 18-24 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

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
S. Typhimurium	ATCC 14028	35-37°C / 18-24H / A	good growth
<i>E. coli</i>	ATCC 25922	35-37°C / 18-24H / A	good growth
<i>Y. enterocolitica</i>	ATCC 23715	29-31°C / 18-24H / 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 Nutrient Agar is tested for productivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by a quantitative method with the target strains *E. coli* ATCC 25922, *S. Typhimurium* ATCC 14028, *Y. enterocolitica* ATCC 23715; Nutrient Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 35-37°C (*E. coli*, *S. Typhimurium*) and at 29-31°C (*Y. enterocolitica*) for 18-24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio ($Pr = CFU_{TB} / CFU_{RB}$) is calculated. If Pr is ≥ 0.7 the results are considered acceptable and conform to the specifications.

Productivity is also tested by semi-quantitative ecometric technique with *E. faecalis* ATCC 19433 and *S. aureus* ATCC 25923. After incubation at 35-37°C for 18-24 hours the amount of growth is evaluated and recorded. All strains show a good growth, comparable with the Reference Batch.

13 - LIMITATIONS OF THE METHOD

- In clinical microbiology, this culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, 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 Material 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 does not 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 inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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 for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

According to MacFaddin the self-prepared plates can be stored at +2°C /+8°C for up to 6-8 weeks while the tubed and bottles media can be store +2°C /+8°C for up to 6 months.³

16 - REFERENCES

- ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp.
- ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica.
- MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> Medical Diagnostic Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/06
Revision 2	Update of "intended use", "test procedure", "precautions and warnings" and "storage conditions and shelf life"	2022/05
Revision 3	Update of chapters 1, 11, 14, 15, 16	2023/03

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

