

**INSTRUCTIONS FOR USE****NUTRIENT AGAR**

Ready-to-use plates

*Salmonella Typhimurium* on Nutrient Agar**1 - INTENDED USE**

*In vitro* diagnostic device. 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 \***

Beef extract	3 g
Peptone	5 g
Agar	15 g
Purified water	1000 mL

\*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 10273. 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 - PHYSICAL CHARACTERISTICS**

Prepared plates appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

**5 - MATERIALS PROVIDED - PACKAGING**

Product	Type	REF	Pack
Nutrient Agar	Ready-to-use plates	541810	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

**6 - MATERIALS REQUIRED BUT NOT PROVIDED**

Incubator, laboratory equipment as required, sterile loops, swabs, ancillary culture media and reagents for the identification of the colonies.

**7 - 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 samples.

**8- 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.

**9- 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.

**10 - 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>S.Typhimurium</i>	ATCC 14028	35-37°C / 18-24H / A (34-38°C acc. to ISO 11133) <sup>3</sup>	good growth
<i>E.coli</i>	ATCC 25922	35-37°C / 18-24H / A (34-38°C acc. to ISO 11133) <sup>3</sup>	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



**11 - PERFORMANCES CHARACTERISTICS**

Prior to release for sale a representative sample of all lots of ready to use plates of Nutrient Agar and of the raw material used for the production of prepared plates (dehydrated Nutrient Agar REF 401810) are 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  $\geq 0,7$  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.

**12 - LIMITATIONS OF THE METHOD**

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, 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.
- 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.

**13 - 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.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each 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.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website [www.biolifeitaliana.it](http://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.

**14 - STORAGE CONDITIONS AND SHELF LIFE**

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).

**15 - REFERENCES**

1. 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.
2. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica.
3. ISO 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media.

**TABLE OF APPLICABLE SYMBOLS**

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

**REVISION HISTORY**

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/10
Revision 2	Removal of obsolete classification	2023/03

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

