

NUTRIENT AGAR N°3

Dehydrated culture medium


 Nutrient Agar n° 3: *Salmonella* Typhimurium

1 - INTENDED USE

General purpose medium for the cultivation, sub-culture and purification of colonies of non-fastidious microorganisms to be submitted to confirmatory tests.

2 - COMPOSITION -TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Meat extract	3.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar	11.0 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

Nutrient Agar n° 3 is a culture medium based on meat peptones, used for the cultivation of non-fastidious microorganisms. The formulation of Nutrient Agar n° 3 corresponds to Nutrient Agar included in some ISO Standards for the subculture of colonies to be submitted to biochemical and serological confirmatory tests:

- *Enterobacteriaceae*, according to ISO 21528^{1,2}
- *Salmonella*, according to 6579³
- *Yersinia*, according to ISO 10273⁴

Meat extract and peptone provide essential nitrogen- and carbon-based nutrients and trace elements for the growth of most non-fastidious microorganisms. Sodium chloride is a source of electrolytes and maintains osmotic balance; agar is the solidifying agent.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24 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	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Agar n° 3	Dehydrated medium	4018142	500 g (20.8 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.

8 - SPECIMENS

Generally Nutrient Agar N° 3 is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

9- TEST PROCEDURE

Allow plates or the tubes 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 or over the slanted medium to obtain well isolated colonies.

Routinely, incubate at 37°C or 30°C in aerobic conditions for 24 hours. Use pure cultures for biochemical and serological confirmatory tests.

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	37°C / 24H / A	good growth
E. coli	ATCC	25922	37°C / 24H / A	good growth
Y. enterocolitica	ATCC	23715	30°C / 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 n°3 is tested for productivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by a semi-quantitative ecometric technique with the following strains: *E. coli* ATCC 25922, *S. Typhimurium* ATCC 14028, *Y. enterocolitica* ATCC 23715, *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433. The amount of growth is evaluated after incubation at 37°C (*E. coli*, *S. Typhimurium*, *S. aureus*, *E. faecalis*) and at 30°C (*Y. enterocolitica*) for 24 hours: all strains show 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 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 ISO 21528 and ISO 6579, the self-prepared plates can be stored protected from drying, at 2-8°C for up to four weeks.¹⁻³

15 - REFERENCES

- ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.
- ISO 21528-2:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 2: Colony-count technique.
- 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.

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	2022/11

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

