

NUTRIENT AGAR WITH NaCl

Dehydrated culture medium and ready-to-use plates

1 - INTENDED USE

General purpose medium for the cultivation, sub-culture and purification of colonies of a wide variety of non-fastidious microorganisms.

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

Beef extract	1.0 g
Yeast extract	2.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 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

There are several proposed formulations of Nutrient Agar in the literature. The Atlas and Parks review¹ lists more than 30 of them. Biolife includes 4 formulations in its product portfolio: the classic formulation (Nutrient Agar REF 401810) with 3 g/L beef extract and 5 g/L meat peptone, Nutrient Agar No. 3 (REF 401814) in which NaCl 5 g/L is added to the classic formulation, Nutrient Agar n°2 (REF 401813) which originates from Nutrient Broth n°2 supplemented with 15 g/L of agar and containing 10 g/L of beef extract, 10 g/L of meat peptone and 5 g/L of NaCl and finally Nutrient Agar w/NaCl (REF 401811). Depending on their operational needs, the end users will be able to choose the formulation they consider most appropriate.

The formulation of Nutrient Agar W/NaCl corresponds to Nutrient Agar recommended by ISO 16266² for the subculture of colonies of suspected *Pseudomonas aeruginosa* to be submitted to confirmatory tests.

This medium may be enriched with other ingredients such as blood, serum, sugars, etc., for specific purposes.

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, maintains osmotic balance and prevents haemolysis when blood is added; agar is the solidifying agent.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28 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	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Agar W/NaCl	Dehydrated medium	4018112	500 g (17.8 L)
Nutrient Agar W/NaCl	Ready-to-use plates	541811	2 x 10 plates ø 90 mm

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 W/NaCl 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 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
<i>P. aeruginosa</i>	ATCC 10145	37°C / 24H / A	good growth
<i>S. aureus</i>	ATCC 25923	37°C / 24H / A	good growth



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

12 - PERFORMANCE CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Nutrient Agar with NaCl are 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, *P. aeruginosa* ATCC 10145, *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433. The amount of growth is evaluated after incubation at 37°C for 24 hours.: all strains show a good growth.

Moreover, the productivity is tested with the medium supplemented with 5% of defibrinated sheep blood with the following strains: *S. pyogenes* ATCC 19615, Group B *Streptococcus* ATCC 12386, Group C *Streptococcus* ATCC 12388. After incubation at 37°C for 24 hours the tested strains exhibit a good growth with beta-haemolysis.

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.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- 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

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 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 16266 the self-prepared plates can be stored in the dark protected from desiccation at 2-8 °C for up to 1 month.²














Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+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

- Atlas R, Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997.
- ISO 16266:2006 Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane filtration.

TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature limitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	 For single use only

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.