# YEAST NITROGEN BASE

**Dehydrated culture medium**

## 1 - INTENDED USE

For the classification of yeasts based on the oxidative utilisation of the carbon containing compounds.

## 2- COMPOSITION

### TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

| | |
|---|---|
| **Nitrogen source** | |
| Ammonium sulphate | 5.00 g |
| **Amino acids** | |
| L-Histidine | 10.00 mg |
| LD-Methionine | 20.00 mg |
| LD-Tryptophan | 20.00 mg |
| **Vitamins** | |
| Niacin - | 0.40 mg |
| P-aminobenzoic acid | 0.20 mg |
| Pyridoxine HCl | 0.40 mg |
| Riboflavin | 0.20 mg |
| Thiamine HCl | 0.40 mg |
| Calcium pantothenate | 0.40 mg |
| Inositol | 2.00 mg |
| Biotin | 20.00 µg |
| Folic acid | 2.00 mg |
| **Trace elements** | |
| Boric acid | 0.50 mg |
| Potassium iodide | 0.10 mg |
| Ferric chloride | 0.20 mg |
| Manganese sulphate | 0.40 mg |
| Sodium molybdate | 0.20 mg |
| Zinc sulphate | 0.40 mg |
| Copper sulphate | 40.00 µg |
| **Salts** | |
| Potassium dihydrogen phosphate | 0.85 g |
| Dipotassium hydrogen phosphate | 0.15 g |
| Magnesium sulphate | 0.50 g |
| Calcium chloride | 0.10 g |
| Sodium chloride | 0.10 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

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom with great industrial importance and pathogenic implications. Primary identification of yeasts is achieved by an assortment of morphological, biochemical and physiological methods. The carbohydrate assimilation technique remains one of the most common and widely used for the definitive identification of yeasts.

Yeast Nitrogen Base is prepared according to the formulation devised by Wickerham[1-3] and modified by Van der Walt[4]. It containing all the growth factors for the yeasts, with the exception of the carbon source. It is suitable for the classification of the yeasts on the basis of the oxidative utilisation of the carbon containing compounds. The results are evident by growth in the liquid medium which is used for assimilation. Yeast Nitrogen Base supplemented with 13.0 g/L of agar, prepared according to Wickerham and Burton's formulation[3], may be used in an auxanographic technique for determining patterns of carbohydrate assimilation.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 6.7 g in 100 mL of cold purified water and sterilise by filtration. The solution will be 10X strength; for use dilute 1:10 with a sterile solution of the chosen carbohydrate: dissolve 0.5 g of the carbohydrate in 90 mL of purified water, sterilise by filtration and aseptically add 4.5 mL of this solution to 0.5 mL of Yeast Nitrogen Base.

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | white, fine, homogeneous, free-flowing powder |
| Solution and prepared tubes appearance | colourless, clear |
| Final pH at 20-25 °C | 5.6 ± 0.2 |

## 6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Yeast Nitrogen Base | Dehydrated medium | 4022552 | 500 g (74.5 L) |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator and laboratory equipment as required, sterile loops and swabs, tubes, flasks, Erlenmeyer flasks, carbohydrates, ancillary culture media and reagents.

## 8 - SPECIMENS

Pure cultures of yeasts.

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## 9 - TEST PROCEDURE

1. Inoculate the tubed medium very lightly with the test organism.
2. Incubate at 25°C for 6-7 days or, if necessary, for 20-24 days. After incubation shake the tubes and read for growth.

## 10 - READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.
Observe the growth of the yeasts by placing the tubes against a white card where black lines (thickness: 3-4mm) have been drawn. If the lines are poorly visible through the culture the test is positive. The yeast growth is often yellow because of the presence of riboflavin.
Refer to the literature for yeast identification schemes based on the results of the carbohydrate assimilation test.[5]

## 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 is listed a test strain useful for the quality control of Yeast Nitrogen Base supplemented with glucose.

| CONTROL STRAIN | INCUBATION T°/ T / ATM | EXPECTED RESULTS |
|---|---|---|
| *C. albicans* ATCC 18804 | 25°C /72h /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 Yeast Nitrogen Base supplemented with suitable carbohydrate solutions is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by inoculating the tubed medium supplemented with mannose, rhamnose, sucrose and lactose with an appropriate decimal dilution of test organisms, incubating at 25°C for 72 hours and recording the tubes showing growth in Reference Batch ($Gr_{RB}$) and in Test Batch ($Gr_{TB}$). Productivity is tested with the following target strains: *C. tropicalis* NCPF 8841, *C. intermedia* CBS572 *C. albicans* ATCC 18804, *S. cerevisiae* ATCC 9763. The tested strains exhibit carbohydrate assimilation results in accordance with specifications and comparable in the Test Batch and Reference Batch.

## 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.
- 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 +2°C /+8°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 and the storage method (temperature and packaging).

## 15 - REFERENCES

1. Wickerham LJ. A simple technique for the detection of melibiose-fermenting yeasts. J Bacteriol 1943; 46:501-505.
2. Wickerham LJ. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J Bacteriol 1946; 52:293-301.
3. Wickerham LJ, Burton KA. Carbon assimilation tests for the classification of yeasts. J Bacteriol 1948; 56:363-371.
4. Van der Walt J P. Criteria and methods used in classification. In: «The Yeasts» ed. Lodder, J. ch.2, pp.84-113. Amsterdam: North Holland. 1971.
5. Borman AM, Johnson EM. Candida, Cryptococcus and other yeasts of medical importance. In Carroll KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Vol 2, 2019. Washington, DC: American Society for Microbiology.

**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 2 | Updated layout and content | 2022/12 |

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

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