WORT AGAR BASE

Dehydrated culture medium

1 - INTENDED USE

For the cultivation of a wide variety of yeasts and filamentous fungi

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

Malt extract	15.0 g
Peptone	0.78 g
Maltose	12.75 g
Dextrin	2.75 g
Dipotassium phosphate	1.0 g
Ammonium chloride	1.0 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

Wort Agar Base is equivalent to the medium described by Parfitt[1] and is suitable for the cultivation and enumeration of fungi, especially yeasts, in butter, sugar and syrups, in lemonades and more generally in sweet or soft drinks.
Several modifications of the wort agar medium have been proposed for specific purposes.[2] According to Rapp[2], addition of certain indicator dyes allows differentiation between yeast and bacterial colonies. Scarr[3] employed a modified Wort Agar ('osmophilic agar') for the examination of sugar products for osmophilic yeasts. Wort or hopped-wort agar with monoiodacetic acid according to Šilhánková[4] is suitable for detection of non-*Saccharomyces* wild yeast; furthermore, monoiodacetic acid suppresses the growth of most bacteria. Wort Agar containing copper sulphate is also used for wild yeast assessment. Wort Agar supplemented with $CuSO_4$ (0.55 g/l) allowed for the growth of non-*Saccharomyces* wild yeasts.[6] Lowering the concentration to 0.2 g/L enables detection of some *Saccharomyces* wild yeasts.[7] According to Röcken et al.[8] incubation of Wort Agar at pH 4.5 at 37 °C is recommended for the detection of amylolytic yeast ("*S. diastaticus*") in bottom-fermenting yeast. The medium, which duplicates the composition of natural wort, contains peptone and malt extract which provide the growth factors for mycological growth. Maltose and dextrin are the fermentable carbohydrates and a source of carbon and energy. The medium acidity (pH 4.8) is favourable for yeast growth and inhibitory to most bacteria. For increasing the selective properties, the pH may be decreased to 3.5 by adding tartaric or lactic acid. Dipotassium phosphate buffers the medium while glycerol, added to the medium base, reduces the water activity.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 48.28 g in 1000 ml of cold purified water and add 2.35 mL of glycerol (REF 421015); heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Mix well and pour into sterile Petri dishes. Prolonged or excessive heating will diminish the gel strength of the agar.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark yellow, limpid
Final pH at 20-25 °C	4.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Wort Agar base	Dehydrated medium	4022032	500 g (10.3 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, sterile Petri dishes, cycloheximide, carbonato di sodio, ancillary culture media and reagents.

8 – SPECIMENS

Butter, sugar and syrups, lemonades and more generally sweet or soft drinks. Refer to applicable International Standards and regulations for the collection of food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE

Yeasts and moulds enumeration in butter.

1. Prepare the initial suspension of the sample and the decimal dilutions with quarter-strength Ringer solution.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of Wort Agar, cooled to approximately 47°C into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Incubate for 5 days at 25°C

10 - READING AND INTERPRETATION

Enumerate the number of colonies of yeasts and moulds per plate and calculate the microbial count.

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. cerevisiae ATCC 9763	25°/ 72 H-A	good growth
S. aureus ATCC 25923	25°/ 72 H-A	growth partially inhibited

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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 Wort Broth Base supplemented with glycerol is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is assessed by a quantitative test with the following strains: *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 18804. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 25°C for 72 hours. The colonies are enumerated on both batches and the productivity ratio (Pr) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications. Moreover, the productivity is tested by modified Miles-Misra technique with *A. brasiliensis* ATCC 16404.After incubation the strain exhibits good growth with typical colonies.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strain *S. aureus* ATCC 25923. The growth of the non-target strain is partially inhibited after incubation at 25°C for 72 hours

13 – LIMITATIONS OF THE METHOD

- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

14 - 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.
- 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 Sheet 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.

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 the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. Parfitt EH. J Dairy Sci. 1933; 16: 141-147.
2. Matoulková D, Kubizniaková P. Brewing Microbiology – Wild Yeasts and Methods of Their Detection. Kvasny Prum. 2013;59: 246–257
3. Rapp, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. Milchwiss. 1974; 29:341-344.
4. Scarr MP J. Sci. Food Agric . 1959; 10(12):678-681.
5. Šilhánková L. A new method of quantitative determination of contamination of baker's yeast by wild yeasts or by dissociation forms of the production culture. Folia Microbiol. 1962; 7: 255–256.
6. Lin Y. Formulating and testing of cupric sulphate medium for wild yeast detection. J. Inst. Brew. 1981;87: 151–154.
7. Taylor GT, Marsh AS. MYGP + copper, a medium that detects both Saccharomyces and non-Saccharomyces wild yeast in the presence of culture yeast. J Inst Brew 1984; 90: 134–145.
8. Röcken W, Schulte S. Nachweis von Fremdhefen. Brauwelt 1986; 41: 1921–1927.

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	2022/08

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