



MALT AGAR

Dehydrated culture medium

1 - INTENDED USE

For the enumeration of yeasts and moulds in cosmetic and food samples and for cultivating yeast and mould stock cultures.

2 – COMPOSITIONS

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

Malt extract	30 g
Agar	17 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

Traditionally acidified media based on malt extract have been used to enumerate yeasts and moulds in different commodities. A medium with 30 g/L of malt extract and agar is known as malt extract agar and is recommended by APHA¹ for the detection and enumeration of heat-resistant moulds in foods. The same medium supplemented with chlortetracycline HCl, is recommended by FDA-BAM² (Medium M93) for the enumeration of yeasts and moulds in cosmetics. Malt Agar is included in Official Methods of Analysis of AOAC International.³ The medium is used for cultivation and maintenance of yeast and mould stock cultures.

Malt Agar contains malt extract, which provides carbon, protein and nutrient sources required for the growth of yeasts and moulds and agar as the solidifying agent. The acidic pH restricts the bacterial growth.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 47 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. If required, add 40 mg/L of chlortetracycline HCl (REF 4240024) after autoclaving. To prepare slants dispense 5-6 mL of boiled medium into 16 x 125 mm screw-cap, loosely cap tubes and sterilize as above. After autoclaving lay tubes in a slanting position and let them solidify.

When the user's procedures require an acidified medium, melt the sterilised medium in boiling water and acidify to pH 4.5 with sterile tartaric acid. To preserve the solidifying properties of the agar do not heat after the addition of tartaric acid.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear or slightly opalescent
Final pH at 20-25 °C	5.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Malt Agar	Dehydrated medium	4016452	500 g (10.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, pipettes and spreaders, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, sterile Petri dishes, chlortetracycline HCl (Dermatophyte Antimicrobial Supplement REF 4240024), tartaric acid, ancillary culture media and reagents.

8 – SPECIMENS

Foods, animal feeding stuffs and other samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Prepare suitable decimal dilutions of the samples.

Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to solidify.

Alternatively, directly inoculate the agar plates using surface spread technique with 0.1 or 0.2 mL of decimal dilutions.

Invert the plates and incubate at 22°C for 5-7 days.

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies

Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

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>Saccharomyces cerevisiae</i> ATCC 9763	25°C/ 72h/A	good growth
<i>Aspergillus brasiliensis</i> ATCC 16404	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 Malt Agar is tested for productivity and selectivity by comparing the results with a Reference Batch.





Productivity is tested by semi-quantitative ecometric method with the target strains *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 18804, *P. chrysogenum* ATCC 10106, *A. brasiliensis* ATCC 16404. The plates are inoculated by surface spreading technique with decimal dilutions in saline of a colonies' suspension and incubated at 25 °C for 72 hours in air. Target strains exhibit good growth with typical colonies. The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. coli* ATCC 25922. The growth of the non-target strain is partially inhibited.

13 – LIMITATIONS OF THE METHOD

- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.⁴
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.⁴
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.⁴
- It is recommended that identification testing be performed on isolates, from pure culture.

14 - 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.

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/flasks) and the applied storage conditions (temperature and packaging). According to MacFaddin, the plated medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks while the tubed medium at +2°C/+8°C for 6 months.⁵

16-REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 23 Microbiological Methods for Cosmetics. Rev December 2021
3. Official methods of analysis of AOAC International, 18th ed. 2007. AOAC International, Gaithersburg, Md.
4. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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	2022/10

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

