MALT EXTRACT AGAR

Dehydrated culture medium

1 - INTENDED USE

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

2 – COMPOSITIONS

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

Maltose	12.5 g
Dextrin	2.5 g
Glycerol	1.0 g
Peptocomplex	2.6 g
Agar	17.0 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

Traditionally acidified media based on malt and malt extracts have been used to enumerate yeasts and moulds in different commodities. Malt Extract Agar is recommended by APHA[1] for the enumeration of yeasts and moulds in water samples and for purifying yeast isolates and studying yeast species in various tests; it is also useful for maintaining stock cultures.
Malt Extract Agar contains maltose as an energy source. Dextrin, derived from starch, and glycerol are carbon sources. Peptocomplex provides nitrogen and minerals for microbial growth. The acidic pH restricts the bacterial growth.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 35.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 115ºC for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. 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.
Excessive heating can decrease the gelling properties of the agar. If a medium with a higher gel strength is desired, add 5 g/L agar to the base medium before autoclaving.
Prolonged thermostating time can lead to the formation of insoluble white precipitates. It is advisable to keep the medium stirring and dispense onto the plate as soon as possible

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, opalescent
Prepared plates appearance	whitish, slightly opalescent
Final pH at 20-25 °C	4.7 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Malt Extract Agar	Dehydrated medium	4016552	500 g (14 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, ancillary culture media and reagents.

8 – SPECIMENS

Waters, foods 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
Saccharomyces cerevisiae ATCC 9763	25°C/72h/A	good growth
Aspergillus brasiliensis ATCC 16404	25°C/72h/A	good growth

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

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12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Malt Extract 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.[2]
- 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.[2]
- 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.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, 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.
- 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.

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.[3]

16-REFERENCES

1. APHA Standards Methods for the Microbiological of Water and Wastewater. American Public Health Association, Washington D.C. 23rd, 2017.
2. 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.
3. 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
Revision 3	Content update	2024/10

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

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