

# YEAST EXTRACT AGAR

## Dehydrated and ready-to-use culture medium

### 1 - INTENDED USE

A highly nutritive medium for plate count of organisms in water samples.

### 2 – COMPOSITION\*

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

#### DEHYDRATED AND READY-TO-USE MEDIUM

Tryptone	6 g
Yeast extract	3 g
Agar	15 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

Yeast Extract Agar is prepared according to the formula described by Windle Taylor<sup>1</sup> for the plate count of microorganisms in water. Yeast Extract Agar is recommended by ISO 6222<sup>2</sup> for the enumeration of aerobic bacteria, yeasts and moulds in water by pour plate method. Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Agar is the solidifying agent.

### 4A - DIRECTIONS FOR DEHYDRATED MEDIUM

Suspend 24 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

### 4B - DIRECTIONS FOR READY TO USE FLASKS/TUBES

Liquefy the contents of the flask/tube in an autoclave set at  $100 \pm 2^\circ\text{C}$  or in a temperature-controlled water bath ( $100^\circ\text{C}$ ). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale yellow, clear
Final pH at 20-25 °C	$7.2 \pm 0.2$

### 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Yeast Extract Agar	Dehydrated medium	4022752	500 g (20.8 L)
Yeast Extract Agar	Ready to use medium in plates	492275	3 x 10 plates ø 55 mm
Yeast Extract Agar	Ready to use medium in tubes	552275	20 x 15 mL
Yeast Extract Agar	Ready to use medium in flasks	5122752	6 x 100 mL
		5122753	6 x 200 mL

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

### 8 – SPECIMENS

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

### 9 - TEST PROCEDURE

- Using a sterile pipette, dispense a volume not exceeding 2 mL of test sample and/or its dilutions, into an empty Petri dish and mix with 15-20 mL of molten Yeast Extract Agar pre-cooled to 44-46°C.
- Invert the plates and incubate one set under aerobic conditions at  $36 \pm 2^\circ\text{C}$  for  $44 \pm 4$  hours; incubate the other set at  $22 \pm 2^\circ\text{C}$  for  $68 \pm 4$  hours.

### 10 - READING AND INTERPRETATION

For each incubation temperature, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per millilitre of the test sample.

### 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>B.subtilis</i> ATCC 6633	34-38°C/44 H-A	good growth
<i>A.brasiliensis</i> ATCC16404	20-24°C/72 H-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 Yeast Extract Agar (Test Batch:TB) are tested for productivity by comparing the results with a previously approved Reference Batch (RB).

The productivity is tested by a quantitative method with the following bacterial strains *E. coli* ATCC 25922, *B. subtilis* ATCC 6633 and the following mycological strains: *C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16404. The plates are inoculated by pour plate technique with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 44 hours (bacterial strains) and at 22°C for 72 hours (mycological strains). The colonies are enumerated on both media and the productivity ratio (Pr: CFU<sub>TB</sub>/CFU<sub>RB</sub>) is calculated. If Pr is ≥ 0.7 the results are considered acceptable and conform to the specifications.

## 13-LIMITATIONS OF THE METHODS

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.<sup>3,4</sup>
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts.<sup>3,5</sup>
- Increasing the holding time of the dilutions in the diluent leads to higher count.<sup>3,6</sup>
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.<sup>3</sup>

## 14 - PRECAUTIONS AND WARNINGS

- This culture medium is for Laboratory use 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](http://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 or tubes to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks or tubes into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle or tubes.
- Once the bottled or tubes medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use medium in tubes and flasks are sterilised by autoclaving.
- 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](http://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

### Ready to use plates

Upon receipt, store plates in their original pack at 2-8°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).

### Ready-to-use medium in flasks and tubes

Upon receipt, store flasks/tubes in their original pack at 2-8°C away from direct light. If properly stored, the flasks/tubes may be used up to the expiration date. Do not use the flasks/tubes beyond this date. Flasks/tubes from opened secondary packages can be used up to the expiration date. Opened flasks/tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks/tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

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


















### 16 – REFERENCES

1. Windle Taylor E. (1958) 'The Examination of Waters and Water Supplies', 7th ed., Churchill Ltd., London, pp. 394-398 and 778.
2. ISO 6222: 1999 Water quality - Enumeration of culturable microorganisms-Colony count by inoculation in a nutrient agar culture medium
3. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
4. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
5. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
6. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130

### 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.

