



## CHLORAMPHENICOL GLUCOSE YEAST EXTRACT AGAR

Dehydrated and ready-to-use culture medium

### 1 - INTENDED USE

Selective medium for the isolation and enumeration of yeasts and moulds in milk, dairy products and other foodstuffs.

### 2 - COMPOSITION - TYPICAL FORMULA \*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	5.0 g
Glucose	20.0 g
Chloramphenicol	0.1 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

Antibiotic supplemented media has been found superior to acidified media for the enumeration of yeasts and moulds in foodstuffs: they are less inhibitory to injured cells, more effective in inhibiting bacterial growth and less likely to cause precipitation of food particles because of their higher pH.<sup>1</sup> Chloramphenicol Glucose Yeast Extract Agar is recommended by ISO 6611 and by FIL-IDF for the isolation and enumeration of yeasts and moulds in milk and dairy products.<sup>2</sup> Yeast extract is a source of vitamins, particularly of the B-group and provides the basic nutrients for microbial growth. Glucose is a carbon and energy source. Chloramphenicol is a thermostable broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria

### 4A - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 40.1 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 45-50°C, mix well and distribute in sterile Petri dishes.

### 4B- DIRECTIONS FOR MEDIUM PREPARATION (MEDIUM IN FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle 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, under aseptic conditions.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared flasks appearance	pale yellow, limpid
Final pH at 20-25 °C	6.6 ± 0.2

### 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chloramphenicol Glucose Yeast Extract Agar	Dehydrated medium	4012892	500 g (12,5 L)
		4012894	5 kg (125 L)
Chloramphenicol Glucose Yeast Extract Agar	Ready-to-use flasks	5112892	6 x 100 mL

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

### 8 - SPECIMENS

Milk, dairy products and other foodstuffs. Refer to applicable International Standards and regulations and operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

### 9 - TEST PROCEDURE

Prepare the sample suspension and the further decimal dilutions with the suitable diluent.<sup>2</sup>

Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.

Add to each plate 15 mL of medium pre-cooled to 45°C. Mix the inoculum with the melted agar and leave to solidify.

Incubate the plates in an inverted position at 25°C and enumerate the colonies after 3-5 days.

To prevent the spreading of the colonies, some precautions should be taken such as: 1) an addition of an overlayer of culture medium after solidifying or 2) an addition of a drop of glycerol on filter paper on the lid of the dish.<sup>2</sup>

### 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies, distinguishing yeasts from moulds. Select plates containing 10-150 colonies and count the colonies.

### 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>C. albicans</i> ATCC 10231	25°C/3 days /A	good growth, typical colonies
<i>A. brasiliensis</i> ATCC 16404	25°C/3 days /A	good growth, typical colonies
<i>P. cyclopium</i> ATCC 16025	25°C/3 days /A	good growth, typical colonies





<i>E. coli</i> ATCC 25922	25°C/3 days /A	inhibited
<i>B. subtilis</i> ATCC 6633	25°C/3 days /A	inhibited

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

## 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready to use Chloramphenicol Glucose Yeast Extract Agar are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).

Productivity is tested by quantitative poured plate technique with the following target strains: *C. albicans* ATCC 10231, *S. cerevisiae* ATCC 9763, *A. brasiliensis* ATCC 1604, *P. cyclopium* ATCC 16025. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 25°C for 3-5 days. The colonies are enumerated on both batches and the productivity ratio (Pr) is calculated. If Pr is  $\geq 0.7$  and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications

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 the non-target strains *E. coli* ATCC 25922 and *B. subtilis* ATCC 6633. The growth of the non-target strains is totally inhibited

## 13 - LIMITATIONS OF THE METHOD

- The method described above is not suitable for a large number of thermolabile yeasts in fresh cheeses. In such case the agar surface plating method is preferred.<sup>2</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 Chloramphenicol Glucose Yeast Extract Agar is classified as dangerous. 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 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 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.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving
- 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 inoculated plates 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

### 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).

### Ready-to-use medium in flasks

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












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/flasks) and the applied storage conditions (temperature and packaging).

## 16 - REFERENCES

1. Beuchat LR. Media for detecting and enumerating yeasts and mould. Int J Food Protect 1992; 17:145:58.
2. ISO 6611:2004 [IDF 94:2004] Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees C.



**TABLE OF APPLICABLE SYMBOLS**

 <b>REF</b> or <b>REF</b> Catalogue number	 <b>LOT</b> Batch code	 <b>Manufacturer</b>	 <b>This side up</b>	 <b>Store in a dry place</b>	 <b>Fragile</b>
 <b>Temperature limitation</b>	 <b>Content sufficient for &lt;n&gt; tests</b>	 <b>Consult Instructions for Use</b>	 <b>Use by</b>	 <b>Keep away from direct light</b>	

**REVISION HISTORY**

<b>Version</b>	<b>Description of changes</b>	<b>Date</b>
Revision 1	Updated layout and content	2022/06

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

