

## SUGAR FREE AGAR

Ready to use plates

### 1 - INTENDED USE

For the enumeration of contaminating microorganisms by means of the colony-count technique.

### 2 - COMPOSITION - TYPICAL FORMULA \*

Gelatin peptone	7.5 g
Tryptone	7.5 g
Sodium chloride	5.0 g
Agar	13.0 g
Purified water	1000 mL

\*The formula may be adjusted and/or supplemented to meet the required performances criteria.

### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sugar Free Agar is prepared as recommended by ISO 13559,<sup>1</sup> Manuel Suisse des Denrées Alimentaires<sup>2</sup> the International Dairy Federation<sup>3</sup> and is based on the formulation developed by Ritter and Eschmann.<sup>4</sup>

According to the recommendations of the ISO Standard<sup>1</sup> the medium can be used for the enumeration of contaminating microorganisms by means of the colony-count technique at 30°C and as recommended by International Dairy Federation<sup>3</sup> for the enumeration of psychotrophic and mesophilic Gram-negative bacteria from butter and other dairy products.<sup>3</sup>

The result of the enumeration furnishes an indication of the level of contamination of the sample tested.

Gelatin peptone and tryptone are sources of nitrogen, carbon, and trace elements needed for the microbial growth; sodium chloride maintains osmotic balance; agar is the solidifying agent. This medium does not contain any fermentable carbohydrates, has an alkaline pH and has relatively poor nutrient value allowing the cultivation of the contaminant flora of the food products.

### 4 - PHYSICAL CHARACTERISTICS

Prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2

### 5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sugar Free Agar	Ready-to-use plates	542098	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

### 6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

### 7 - SPECIMENS

Butter and other processed dairy products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.<sup>1,2,3</sup>

### 8 - TEST PROCEDURE

ISO 13359<sup>1</sup> recommends the following technique.

Transfer to each of two prepared plates by means of a sterile pipette 0,1 ml of the initial suspension of the product.

Repeat this operation using further decimal dilutions.

Carefully spread the inoculum as quickly as possible over the surface of the plate, using a sterile spreader.

Leave the plates, with the lids on, for approximately 15 min on the bench to allow absorption of the inoculum into the plates.

Invert the prepared dishes and place them in the incubator set at 30 °C for 72 h ± 2 h.

### 9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Count the colonies after incubation. Count the colonies characteristic for contaminating microorganisms in each dish containing not more than 150 colonies. Do not count pin-point colonies as these are not typical for contaminants.

Information as to the source of contamination may be obtained by examination of the colonies present. It can be valuable, therefore, to record the types of colonies present (e.g., pure or mixed, yeasts, moulds, *Bacillus* sp. etc.).<sup>1</sup>

Contaminating microorganisms as defined by ISO 12259<sup>1</sup>: non-lactic acid bacteria, yeasts and moulds forming countable colonies under the conditions specified in the International Standard.

Note 1 Product-specific lactic acid bacteria will not be detected by this method.

Note 2 In certain types of fermented milks, non-lactic acid bacteria, yeasts or moulds can be part of the microflora contributing to the desired characteristics of the product. In such cases, care should be taken when applying the method here described.

### 10 - 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, it is responsibility of the end-user to perform Quality Control testing 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>E.coli</i>	ATCC 25922	30°C / 72 H / A	good growth
<i>E.faecalis</i>	ATCC 19433	30°C / 72 H / A	good growth

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

### 11 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control only and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each 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.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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.

### 12 - STORAGE CONDITIONS AND SHELF LIFE

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).

### 13 - REFERENCES

1. ISO 13559:2002 Butter, fermented milks and fresh cheese - Enumeration of contaminating microorganisms - Colony-count technique at 30°C.
2. Manuel Suisse des Denrées Alimentaires. 5<sup>e</sup> édition, deuxième volume, Chap. 56. 1988
3. International Dairy Federation (1964). International standard count of contaminating organisms in butter. International Standard FIL- IDF30.
4. Ritter P. and Eschmann K. H., 1966, *Alimenta*, 5 (2): 433.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD In vitro Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

### REVISION HISTORY

Version	Description of changes	Date
Revision 0	First edition	2022/02

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

