SUGAR FREE AGAR BASE
PENICILLIN G 500 IU SELECTIVE SUPPLEMENT
Dehydrated culture medium and selective supplement

1 - INTENDED USE
For the enumeration of microorganisms not related to dairy-products production process.

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

SUGAR FREE AGAR BASE – DEHYDRATED MEDIUM

Gelatin peptone	7.5 g
Tryptone	7.5 g
Sodium chloride	5.0 g
Agar	13.0 g

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

PENICILLIN G 500 IU SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 100mL OF MEDIUM)

Penicillin G sodium salt	500 IU

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
The microbiology of butter and other dairy products reflects the microflora present in milk and water, the sanitary conditions of process equipment, manufacturing environment, and conditions under which the product is stored. A large number of microorganisms may contaminate the dairy products: psychrophilic/psychrotrophic bacteria, mesophilic bacteria, sporeformers, yeasts and moulds. Th estimation of the number of “infective organisms” which are not directly involved in the production process, taken at various stages of processing, is useful in tracing the source of contamination.
Sugar Free Agar Base is based on the formulation of Ritter and Eschmann[1], is free of fermentable carbohydrates, has low nutritive characteristics and is primary used for the enumeration of contaminant microorganisms not directly involved in the production process of butter and dairy products.[2]
Supplemented with penicillin G, Sugar Free Agar can be used for the enumeration of mesophilic aerobic Gram-negative bacteria such as *Pseudomonas, Flavobacterium, Alkaligenes, Aeromonas, Xantomonas, Acinetobacter, Enterobacteriaceae*.[3]
The medium contains peptones with a little nutritive value which provide the growth factors for microbial growth, substantially reducing the growth of microorganisms related to production process, in particular lactobacilli, thus favouring the growth of spoiling microorganisms. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Agar is the solidifying agent.

4 - DIRECTIONS FOR MEDIA PREPARATION
Sugar Free Agar
Suspend 33 g in 1000 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
Sugar Free Penicillin Agar
If a Sugar Free Penicillin Agar is required, subdivide the medium after boiling in 100 mL aliquots and autoclave at 121°C for 15 minutes. Cool to approximately 45°C and add the contents of one vial of Penicillin G 500 IU Selective Supplement (REF 4240050) reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Freeze dried supplement	short, dense, white pellet; clear colourless solution after reconstitution
Final pH at 20-25 °C	7.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sugar Free Agar Base	Dehydrated medium	4020982	500 g (15.1 L)
Penicillin G 500 IU Selective Supplement	Freeze-dried supplement	4240050	10 vials, each for 100 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

8 – SPECIMENS
Butter and other dairy products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE
1. Prepare the initial suspension of the sample and the decimal dilutions with the suitable diluent.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of Sugar Free Agar Base or Sugar Free Agar Base supplemented with penicillin G, cooled to approximately 47°C, into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Incubate at the temperature required by the analysis for detecting psychrophilic/psychrotrophic /mesophilic bacteria (e.g., 30°C for 72 hours, or 48 hours at 35 °C, followed by 48 hours at 20 °C).

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10 - READING AND INTERPRETATION

Enumerate the number of colonies per plate and calculate the microbial count. Do not count pin-point 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
E. coli ATCC 25922	30°/ 24 H-A	good growth
P. aeruginosa ATCC 14207	30°/ 24 H-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 Sugar Free Agar Base supplemented with Penicillin G 500 IU Selective Supplement (REF 4240050) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is assessed by semi-quantitative ecometric technique with the following strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 14207, *A. calcoaceticus* ATCC 19606. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 24 hours. The strains exhibit good growth.
Selectivity is tested 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 following non-target strains: *S. aureus* ATCC 25923 and *E. faecalis* ATCC 19433. The growth of the non-target strain is totally inhibited.

13 – LIMITATIONS OF THE METHOD

- The isolated colonies on the plates should be identified with suitable tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Penicillin G 500 IU Selective Supplement is classified as hazardous. Before use, consult the Material Safety Data Sheets.
- 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 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.
- Be careful when opening the metal ring of the supplement vial to avoid injury.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet 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

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).

Freeze-dried supplement

Upon receipt, store the product in the original package at +2°C / +8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

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. Ritter P., Eschmann KH. 1966, Alimenta, 5 (2): 433.
2. International Dairy Federation: Methods of sampling milk and milk products. International Standard, FIL/IDF 50 B, 1985.
3. Manuel Suisse des Denrées Alimentaires. 5° edition, deuxième volume, Chap. 56. 1988.

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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

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