SPORULATION AGAR AK
SPORULATION BROTH

Dehydrated culture media

1 - INTENDED USE

For the preparation of spore suspensions used to detect antibiotic residues in milk and dairy products.

2 – COMPOSITION*

TYPICAL FORMULAS (AFTER RECONSTITUTION WITH 1 L OF WATER)

SPORULATION AGAR AK

Gelatin peptone	6.0 g
Yeast extract	3.0 g
Tryptone	4.0 g
Beef extract	1.5 g
Glucose	1.0 g
Manganous sulphate	0.3 g
Agar	15.0 g

SPORULATION BROTH

Gelatin peptone	6.0 g
Yeast extract	3.0 g
Tryptone	4.0 g
Beef extract	1.5 g
Glucose	1.0 g
Manganous sulphate	0.3 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sporulation, unique to two bacterial species, *Clostridium* and *Bacillus*, is a process induced by reduced levels of nutrients in the environment or in culture.[1] A specific sporulation medium is required for producing spores from specific group of spore-forming bacteria.
Sporulation Agar AK, also known as AK 2 Agar or USP Antibiotic Medium 32, is a modification of Antibiotic Assay Medium No.1, described by Arret and Kirshbaum[2] for the production of *Bacillus subtilis* spores for rapid disc assay method for detecting penicillin in milk. The medium was also found useful for spore production in *Bacillus megaterium* ATCC 9885.[3,4]
Gelatin peptone, beef extract and tryptone provide nitrogen, amino acids and trace elements for microbial growth. The yeast extract is a source of vitamins, particularly of the B-group. Glucose is a fermentable carbohydrate and a source of energy. Manganese ions are necessary for the activity of the enzyme phosphoglycerate phosphomutase involved in the sporification process.[5]
Sporulation Broth is prepared with the same formulation of Sporulation Agar AK with the omission of agar. It can be used for sporification in a liquid culture medium or added to agar for growth on the medium surface.

4A – SPORULATION AGAR AK: DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and autoclave at 121° C for 15 minutes.

4B – SPORULATION BROTH: DIRECTIONS FOR MEDIUM PREPARATION

Suspend 15.8 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and autoclave at 121° C for 15 minutes. If required, add 15 g/L of agar (REF 411030) before autoclaving.

5 - PHYSICAL CHARACTERISTICS

Dehydrated media appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	6.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sporulation Agar AK	Dehydrated medium	4020702	500 g (16.2 L)
Sporulation Broth	Dehydrated medium	4020712	500 g (31.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, incubator and laboratory equipment as required, Roux bottle, ancillary culture media and reagents.

8 – SPECIMENS

Pure culture of spore-forming bacteria.

9 - TEST PROCEDURE

Preparation of *B. subtilis* spores with Sporulation Agar AK [6]

1. Transfer monthly *B.subtilis* ATCC 6633 culture to fresh slant of Antibiotic Seed Agar A1 (REF 401075)
2. Wash the growth from the slant with sterile physiological saline onto the surface of a Roux bottle containing 300 mL of solidified Sporulation Agar AK.
3. Incubate the bottle aerobically at 35°C for 5 days with caps loosened.
4. Aseptically wash off the resulting growth into 50 mL of sterile physiological saline with the aid of sterile crystal pearls if necessary.
5. Centrifuge and aseptically decant and discard the supernatant.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

6. Resuspend the sediment in few millilitres of sterile saline and heat to 70°C for 30 minutes.
7. The spore suspension can be stored for several months.[6]

Preparation of *B. subtilis* spores with Sporulation Broth[7]

1. For sporulation in the liquid medium, 25 mL of an overnight bacterial culture is added into a 2 L flask containing 250 mL of fresh Sporulation Broth and incubated at 37 °C with vigorous shaking at 200 rpm for at least 3 days, until the fraction of spores/vegetative cells came to a maximum level.
2. Centrifuge, resuspend the sediment and heat the culture as described above.

Consult the references for the test procedures utilizing *B. subtilis*[2] or *B. megaterium*[4] spore suspensions.

10 - Reading and interpretation

The resulting growth on the medium surface is compact and the extent of sporulation must be assessed by microscopic observation.
The resulting growth on the liquid medium is indicated by a varying degree of turbidity, specks and flocculation in the medium. The extent of sporulation must be assessed by microscopic observation.

11 - User quality control

All manufactured lots of the products 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
B. subtilis ATCC 6633	35°/ 5 days/A	Good growth; spores present

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 Sporulation Agar AK and Sporulation Broth supplemented with 15 g/L of agar, are tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is assessed by the Roux bottle sporulation test with *B. subtlis* ATCC 6633. After incubation at 37°C for 5 days, the extent of sporulation is assessed by microscopic observation and compared with the sporulation obtained in the reference medium. At the microscope, a comparable number of spores is observed in the Test Batch and the Reference Batch.

13 - Precautions and warnings

- Sporulation Agar AK and Sporulation Broth 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Sporulation Agar AK and Sporulation Broth contain 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 products do not contain any transmissible pathogen. Therefore, it is recommended that the culture media 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 media and the sterilized media inoculated with microbial strains in accordance with current local legislation.
- Do not use the culture media as active ingredients for pharmaceutical preparations or as production materials 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.
- 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.

14 - 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 and the applied storage conditions (temperature and packaging).

15 - References

1. Driks A. Overview: development in bacteria: spore formation in Bacillus subtilis. Cell Mol Life Sci 2002; 59: 389-391.
2. Arret B, Kirshbaum A. A rapid disc assay method for detecting penicillin in milk. J Milk Food Tec. 1959; 22:329.
3. Verma N, Singh NA, Kumar N, Raghu HV. Screening of different media for sporulation of Bacillus megaterium. Int J Microbiol Res and Rev 2013;1: 68-73.
4. APHA Standard Methods for the Examination of Dairy Products. American Public Health Association. Washington, D.C. 15th ed.1985
5. Vasantha N, Freese E. The role of manganese in growth and sporulation of Bacillus subtilis. J Gen Microbiol 1979; 112:329-36.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
7. Li L, Jin J, Hu H, Deveau IF, Foley SL, Chen H. Optimization of sporulation and purification methods for sporicidal efficacy assessment on Bacillus spores. J Ind Microbiol and Biotechnol 2022; 49:1-10.

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 1	Updated layout and content	2022/12

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

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E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it