ChromArt

CHROMOGENIC B.CEREUS AGAR BASE
CHROMOGENIC B.CEREUS SUPPLEMENTS

Dehydrated culture medium, selective supplement and enrichment

1 - INTENDED USE

For the enumeration of *Bacillus cereus* Group in foods and environmental samples.

2 - COMPOSITION*

BACILLUS CEREUS AGAR BASE
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptones	20.0 g
Sodium chloride	5.0 g
Chromogenic mix	0.2 g
Agar	15.0 g

CHROMOGENIC B.CEREUS SELECTIVE SUPPLEMENT (4240090S)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobial mix	75 mg

CHROMOGENIC B.CEREUS ENRICHMENT SUPPLEMENT (4240090E)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Phospholipids	10 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

Bacillus cereus is a group of ubiquitous facultative anaerobic, sporeforming, Gram-positive rods, commonly found in soil, on vegetables, and in many raw and processed foods. The group consists of at least eight closely related species: *B.cereus*, *B.thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, *B.anthracis*, and *B. toyonensis.*[1]
B. cereus food poisoning may occur when foods, such as cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts, are prepared and held without adequate refrigeration for several hours before serving, with *B. cereus* reaching >10^6 cells/g.[2]
Bacillus cereus may cause an emetic or a diarrhoeal type of food-associated illness; the emetic disease is a food intoxication caused by cereulide, a small ring-formed dodecadepsipeptide while the diarrhoeal syndrome is an infection caused by vegetative cells, ingested as viable cells or spores, thought to produce protein enterotoxins in the small intestine.[3]
The current method recommended by ISO Standards for the enumeration and identification of *B. cereus* includes growth on MYP and PEMBA media.[4,5] Problematic issues with traditional media include a lack of characteristic colony morphology, masked by the presence of background flora, such as *Bacillus* species other than *B. cereus* group and *S. aureus*.[2]
Chromogenic B.Cereus Agar inhibits the growth of background flora and includes a specific chromogenic compound for the detection of β-glucosidase enzyme and a substrate for the detection of phospholipase. Colonies of *B. cereus* and *B. cereus* Group are blue-green with a typical zone of precipitation. The antimicrobial mix strongly reduces the background Gram negative and Gram positive flora and allows to isolate *B.cereus* Group often in pure culture. Biochemical testing is necessary to delineate to the species level.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20.1 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 42-45 °C and, under aseptic conditions, add the following supplements: the contents of one vial of Chromogenic B. Cereus Enrichment Supplement (4240090E) and the contents of one vial of Chromogenic B.Cereus Selective Supplement (4240090S), reconstituted with 5 ml of sterile purified water. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared plates appearance	yellow, opaque
Freeze-dried selective supplement	dense, white, pellet
Reconstituted selective supplement	homogeneously cloudy solution
Enrichment supplement appearance	yellow suspension, homogenously opaque
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Chromogenic B.Cereus Agar Base	Dehydrated medium	4080202	500 g (12.4 L)
Chromogenic B.Cereus Supplements	Freeze-dried and liquid supplements	4240090	4 + 4 vials (each vial is for 500 mL of medium base)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

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8 - SPECIMENS

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[2,4,5]

9 - TEST PROCEDURE

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned.
Distribute 0.1mL of test sample if the product is liquid, or of the initial suspension if solid onto the surface of two agar plates (90mm). Repeat the procedure using further decimal dilutions.
If low number of *B.cereus* is expected, distribute 1mL of test sample if the product is liquid or 1mL of the initial suspension if solid to each of two agar plates (140 mm) or over the surface of three 90 mm plates.
Incubate at 30°C ± 1°C in aerobic conditions for 24 ± 2 hours. If colonies are not visible incubate the plates for further 24 hours before counting.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count the presumptive *B. cereus* Group colonies in the plates with less than 150 colonies, that have the following characteristics: large, blue-green and generally surrounded by a zone of precipitation (indicating the production of phospholipase).
According to ISO Standards[4,5] typical and atypical colonies must be confirmed by means of the haemolysis test on sheep blood agar (+): select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar, incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.
According to FDA-BAM typical and atypical colonies must be confirmed with Gram staining (*B. cereus* will appear as large Gram-positive bacilli in short-to-long chains; spores are ellipsoidal, central to subterminal, and do not swell the sporangium), Phenol red glucose broth (+), Nitrate broth (+), Modified VP medium (+), Tyrosine agar (+), Lysozyme broth (+)
Thanks to the high selectivity of Chromogenic B.Cereus Agar, the confirmation tests on typical colonies could be omitted.

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
B. cereus	ATCC 11778	30 °C / 24 h - A	Blue-green colonies with opaque halo
B. subtilis	ATCC 6633	30 °C / 48 h - A	Inhibited
E. coli	ATC 25922	30 °C / 48 h - 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 Chromogenic B.Cereus Agar Base and supplements (Test Batch:TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch and Tryptic Soy Agar (TSA).
Productivity is tested by a quantitative test with the target strains *B. cereus* ATCC 11778 and ATCC 14579: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 24 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr:CFU_{TB}/CFU_{TSA}$) is calculated. If *Pr* is ≥ 0.5 and if the colonies morphology and colour are typical (green-blue colonies with opaque halo) the results are considered acceptable and conform to the specifications. Furthermore, the productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *B. cereus* ATCC 9139 and *B. thuringiensis* ATCC 10792. The amount of growth and colonies characteristics are evaluated after incubation at 30°C for 24 hours: both strains grow with green-blue colonies with opaque halo.
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. faecalis* ATCC 19433, *E. coli* ATCC 25922, *B. subtilis* ATCC 25923, *L. monocytogenes* ATCC 19433. After incubation at 30°C for 48 hours, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Some strains of *B. cereus* produce only little or no phospholipase. Colonies of these strains will not be surrounded by a precipitation zone.[4]
- *Bacillus megaterium* may grow with blue-green colonies but without opaque halo.[2]
- Some bacteria can also grow as blue-green coloured colonies on the medium but without expression of the phospholipase activity. The absence of the opaque halo will make them easily distinguishable from the *Bacillus cereus*.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements 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 and antibiotics containing supplements must be handled with suitable protection. Chromogenic B.Cereus 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 supplements to avoid injury.
- The selective 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 supplements and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements 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 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 +2°C /+8°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 selective 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).

Liquid supplement

Store until the expiry date stated on the label, at +2°C /+8°C. Do not use beyond this date. Open the liquid enrichment bottle with aseptic precautions and store at +2°C /+8°C until the expiry date if the contents are not fully used.

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

16 - References

1. Ehling-Schulz M, Koehler TM, Lereclus D. The Bacillus cereus Group: Bacillus species with Pathogenic Potential. Microbiol Spectr. 2019 May; 7(3): 10.
2. FDA-BAM, Chapter 14: Bacillus cereus. Content current as of: 06/29/2021
3. Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol Rev. 2008 Jul;32(4):579-606
4. ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive Bacillus cereus - Colony-count technique at 30 degrees C.
5. ISO 21871:2006 Microbiology of food and animal feeding stuffs - Horizontal method for the determination of low numbers of presumptive Bacillus cereus- Most probable number technique and detection method.

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	2022/06

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

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