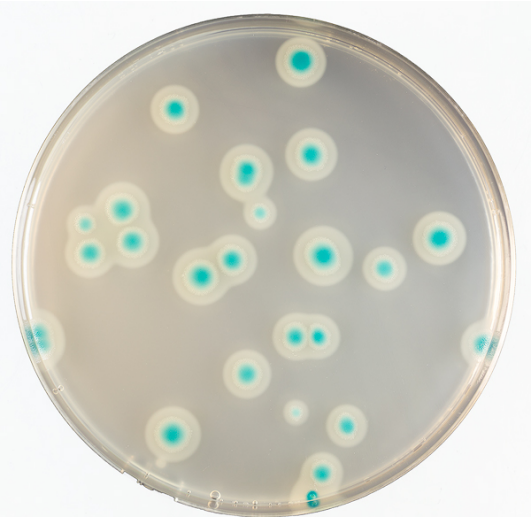
ChromArt

CHROMOGENIC B.CEREUS AGAR BASE
CHROMOGENIC B.CEREUS SUPPLEMENTS

Chromogenic medium base and supplements for the enumeration of *Bacillus cereus* Group in food chain.



Typical *Bacillus cereus* colonies

INTENDED USE

Chromogenic medium base and supplements for the enumeration of *Bacillus cereus* Group in products intended for human consumption and the feeding of animals, and environmental samples in the area of primary production, food production and food handling.

TYPICAL FORMULA OF MEDIUM BASE (PER LITRE) *

Peptones	20,0 g
Sodium chloride	5,0 g
Chromogenic mix	0,2 g
Agar	15,0 g

* Adjusted and/or supplemented to meet performances criteria.

TYPICAL FORMULAS OF SUPPLEMENTS (PER VIAL)

CHROMOGENIC B.CEREUS SELECTIVE SUPPLEMENT (4240090S)

Antimicrobial mix	75 mg

CHROMOGENIC B.CEREUS ENRICHMENT SUPPLEMENT (4240090E)

Phospholipids	10 ml

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.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance: grey, fine, homogeneous, free-flowing powder.
Freeze-dried supplement: white pastille, limpid or slightly opalescent after reconstitution,
Liquid supplement: opaque yellow suspension.
Prepared medium appearance: very pale yellow, opaque.
Final pH of the complete medium at 25°C: 7,2 ± 0,2

MATERIAL PROVIDED

Chromogenic B.Cereus Agar Base in powdered form; Chromogenic B.Cereus Selective Supplement in lyophilised form and Chromogenic B.Cereus Enrichment Supplement in liquid form.

MATERIAL REQUIRED BUT NOT PROVIDED

Autoclave, sterile petri dishes, flasks, loops, ancillary reagents and culture media, incubator, water baths and laboratory equipment.

SPECIMENS

Food products and ingredients intended for human consumption, the feeding of animals and environmental samples in the area of food production and food handling. Follow the recommendations in the current standards to perform specimen collection and preparation.

PRINCIPLE OF THE METHOD AND EXPLANATION

Bacillus cereus is a group of ubiquitous facultative anaerobic sporeforming Gram-positive rods commonly found in soil. *Bacillus cereus* Group includes *B.cereus*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudo-mycoides* and *B. anthracis*. *Bacillus cereus* is responsible for food poisoning, provoked by two toxins, one being heat stable and emetic and the other being thermolable the cause of diarrhoea. The infection is caused by ingestion of foods such as meat, eggs, dairy products, rice and vegetables that are contaminated with *B. cereus,* and have been left at room temperature after cooking. The minimum infective dose is 100 cells/g of food. The diagnosis must be supported by the isolation of *B. cereus* from foodstuffs and faeces using quantitative cultures.
The current method recommended by ISO for the enumeration and identification of *B. cereus* includes growth on MYP medium. Problematic issues with MYP include a lack of characteristic colony morphology and masked by the presence of background flora, such as *Bacillus* species other than *B. cereus* and *S. aureus*.
The medium here described 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 (with the exception of *B. anthracis).* The antimicrobial mix strongly reduce the background Gram negative and Gram positive flora and allow to isolate *B.cereus* Group often in pure culture.

PROCEDURE FOR INOCULATION AND INCUBATION

For the isolation and enumeration of *B. cereus* in foodstuffs the following method should be used:

- 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 (140mm) or over the surface of three 90mm 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.

RESULTS READING AND INTERPRETATION

- 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).
- Select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar in a manner which allows good interpretation of the haemolysis reaction. Incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.

QUALITY CONTROL

The user is responsible for performing the quality control according to national regulations. The following strains are given as an example for the user's quality control:

TEST STRAINS		INCUBATION	SPECIFICATIONS
B. cereus	ATCC 11778	30 °C x 24 h - A	Blue colonies with opaque halo
B. thuringiensis	ATCC 10792	30 °C x 24 h - A	Blue colonies with opaque halo
B. subtilis	ATCC 6633	30 °C x 48 h - A	Inhibited
E. coli	ATC 25922	30 °C x 48 h - A	Inhibited
L. monocytogenes	ATCC 19433	30 °C x 48 h - A	Inhibited

ATCC is a registered trade mark of American Type Culture Collection
A: aerobic incubation

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. *Bacillus thuringensis* often grows with blue-green colonies but without opaque halo.
- Thanks to the high specificity and selectivity of the medium here described, the confirmation tests on typical colonies could be omitted.
- 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*.

WARNING AND PRECAUTIONS

- Before the use of powdered medium base and supplements here described, consult the Material Safety Data Sheets
- Chromogenic B.Cereus Agar Base contains products from animal origin (peptones). Download from the web site www.biolifeitaliana.it the document for Risk Assessment of Products from Animal Origin.

- Chromogenic B.Cereus Agar Base shall be used only with Chromogenic B.Cereus Supplements. Chromogenic B.Cereus Supplements shall be used only for the supplementation of Chromogenic B.Cereus Agar Base.
- The culture medium and supplements here described are for Laboratory use only and should be used by trained Laboratory technicians.
- Sterilize all biohazard waste before disposal.
- Download the Quality Control Certificate from the website www.biolifeitaliana.it

STORAGE

Chromogenic B.Cereus Agar Base: upon receipt store at 2°C to 8°C and protect from the direct light. The expiry date applies to the products in the intact packaging when stored as directed and to the well re-closed bottle.
Chromogenic B.Cereus Supplements : upon receipt store at 2°C to 8°C The expiry date applies to the un-opened vials.

REFERENCES

- ISO7932 :2004 - Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of presumptive Bacillus cereus -- Colony-count technique at 30 degrees C
- ISO 21871:2005 - 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
- Holbrook, A. and Anderson, J.M. (1980), Can. J. Microbial, **26**,753-759
- Weasel, D.A.A., Kaapman. M.J. and Jongerius, E. (1967), App. Microb. **15**, 650
- FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.

ORDERING INFORMATION

Product	Type	Cat. N°	Pack size
Chrom*Art* Chromogenic B.Cereus Agar Base	DCM	4080202	500 g (12,4 L)
Chrom*Art* Chromogenic B.Cereus Supplements	Supplements	4240090	4 + 4 vials (each vial is for 500 ml of medium base)

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