

# Bacillus Cereus Supplement ISO

Cat. 6021

Selective supplement for the isolation and enumeration of Bacillus cereus.

## Practical information

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Applications	Categories
Selective enumeration	Bacillus cereus
Selective isolation	Bacillus cereus

Industry: Clinical / Food

Regulations: ISO 21872 / ISO 7932

## Principles and uses

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Bacillus Cereus Supplement contains polymyxin B, an antibiotic that inhibits most gram-negative bacteria. Polymyxins bind to the cell membrane and disrupt its structure making it more permeable. This antibiotic has little effect on Gram-positive bacteria because of the thick cell wall.

This supplement is used in the following media:

- Bacillus Cereus Selective Agar Base (MYP) (Cat. 1343).
- Bacillus Cereus Selective Agar Base (PEMBA) (Cat. 1125).
- Bacillus Cereus Selective Agar Base (Cat. 1124).

They are selective mediums that are used for the enumeration of presumptive Bacillus cereus, according to ISO 7932 and ISO 21871 respectively.

Bacillus cereus is resistant to certain concentrations of polymyxin, which inhibits the accompanying flora, and it is effective mainly against Gram-negative organisms. Bacillus cereus produces lecithinases. The insoluble degradation products of the lecithin from egg yolk, are accumulated around the Bacillus cereus colonies, forming a white precipitate.

- PEMBA: blue colonies, surrounded by a clear zone of lecithinase activity.
- MYP: brilliant pink opque colonies, with clear lecithinase halo.

## Formula per vial

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Polymixin B (IU)	50000
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## Preparation

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Aseptically reconstitute 1 vial with 6 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 450 ml of Bacillus Cereus Selective Agar Base (Cat. 1124) autoclaved, cooled to 50 °C and supplemented with 50 ml of Egg Yolk Emulsion (Cat. 5152). Mix well and distribute into sterile containers. This supplement can also be added to Bacillus Cereus Agar Base (MYP) ISO 7932 (Cat. 1343).

## Instructions for use

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For the enumeration of presumptive Bacillus cereus according to ISO 7932:

- Transfer 0,1 ml of the test sample or of the initial suspension to each of two agar plates. Make decimal dilutions if necessary.
- When, for certain products, it is desirable to estimate low numbers of B. cereus, the limits of detection may be raised by a factor of 10, by examining 1,0 ml of the test sample if the initial product is liquid, or 1,0 ml of the initial suspension for the other products.
- Distribute the 1 ml of inoculum either on the surface of a large Petri dish (140 mm) or over the surface of three small dishes (90 mm) using a sterile spreader. In both cases, prepare duplicates by using two large plates or six small plates.
- Inoculated plates should be incubated for 18 hours to 24 hours at 30 °C. If colonies are not clearly visible, incubate the plates for an additional 24 hours before counting.

For enumerate low numbers of Bacillus cereus according to ISO 21871:

- Enrichment in selective liquid medium TSPB:  
3 tubes with 10 ml of medium at double concentration.  
3 tubes with 9 ml of the medium at simple concentration.
- Inoculate the double concentration tubes with 10 ml of the initial suspension of the sample to be analyzed, and inoculate the single concentration tubes

with 1 ml of the initial suspension or subsequent dilutions.

- Inoculate at 30 °C for 48±4 hours.

- Shake the tubes gently and inoculate the Selective Agar for Bacillus Cereus (PEMBA) with a sowing handle (10 µl).

- Incubate the plates in inverted position 37 °C for 18-24 hours. If the colonies have not been identified correctly, continue with the incubation for 24 hours more.

- Examine the plates to detect the presence of colonies.

## Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Lyophilized tablet	N/A	Transparent	N/A

## Microbiological test

Incubation conditions: (24-48 h/ 35±2 °C).

Inoculation conditions: Productivity (100±20. Min.50 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU).

Microorganisms	Specification
Bacillus cereus ATCC 11778	Good growth
Escherichia coli ATCC 25922	Total inhibition (0)

## Storage

Temp. Min.:2 °C

Temp. Max.:8 °C

## Bibliography

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD. (2003) Handbook of Culture Media for Food Microbiology. Elsevier Sci. B.V. Amsterdam. The Netherlands.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington DC. USA.
- FIL-IDF 181:1998 Provisional Int. Standard. Dried Milk Products. Enumeration of Bacillus cereus.- Most probable number technique.
- ISO 7932 Standard (2004) 3rd ed. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus. Colony count technique at 30°C.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 21871 Standard (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.
- MOSSEL, D.A.A., KOOPMAN. M.J. & JONGERIUS, E. (1967) Enumeration of Bacillus cereus in foods. Appl. Microbiol. 15:650-653.
- PASCUAL ANDERSON, M<sup>a</sup>.R<sup>a</sup> (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.