Instructions For Use

ENGLISH

Bacillus Cereus Agar (PEMBA)

Selective medium for detection of *Bacillus cereus* according to ISO 21871.

DESCRIPTION

Bacillus Cereus Agar (PEMBA) is a medium used for the selective isolation and enumeration of *B. cereus* from food, animal feed and environmental samples. This medium is not intended for use in the diagnosis of diseases or other conditions in humans.

This medium, Polymyxin pyruvate Egg yolk Mannitol Bromothymol blue Agar, complies to ISO 21871 for the determination of low numbers of presumptive *Bacillus cereus*.

TYPICAL FORMULA* (Per litre of Purified Water)

Enzymatic Digest of Casein	1.0 g
D-Mannitol	10.0 g
Sodium Chloride	2.0 g
Magnesium Sulfate	0.1 g
Disodium Hydrogen Phosphate	2.5 g
Potassium Dihydrogen Phosphate	0.25 g
Sodium Pyruvate	10.0 g
Bromothymol Blue	0.12 g
Polymyxin B Sulfate	100 000 units
Egg Yolk Emulsion	50.0 ml
Agar	15.0 g
Final pH 7.2 ± 0.2 at 25°C	

*Formula may be adjusted and/or supplemented as required to meet performance specifications;

METHOD PRINCIPLE

Enzymatic digesto fo casein provides nitrogen and amino acids. Sodium chloride maintains the osmotic balance of the medium. Mannitol is the carbon source. Magnesium sulfate provides ions, disodium phosphate and monopotassium phosphate constitute the buffer system of the medium. Sodium pyruvate stimulates the growth of microorganisms. Bromothymol blue is the pH indicator. Polymyxin B inhibits the growth of Gram-negative bacteria. The egg yolk emulsion is incorporated to detect the proteolytic activity. Agar is the solidifying agent.

TEST PROCEDURE

Following ISO 21871, streak the enrichment culture onto the surface of the PEMBA plate using an inoculation loop.

Incubate the inoculated plates in inverted position at 37 ± 1°C for 18-24 hours in aerobic conditions. Additional 24 h incubation (also at room temperature) may be necessary to clearly assess colonies.

INTERPRETING RESULTS

Mannitol-fermenting organisms form yellow colonies. Bacteria that produce lecithinase hydrolyze lecithin showing a zone of white precipitate around the colonies.

Bacillus cereus, which is typically mannitol-negative and lecithinase positive, forms turquoise-blue colonies (about 2-5 mm in size) surrounded by a distinct opaque zone of egg yolk precipitation up to 5 mm wide.

Presumptive identification shall be confirmed by haemolysis test on sheep blood agar or, alternatively, by means of a microscopic examination.

STORAGE

Store at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

Avoid quick temperature shifts to prevent condensation.

SHELF LIFE

6 months.

QUALITY CONTROL

Appearance of Medium: Opaque, yellow to yellow-green.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Specification
Bacillus cereus	WDCM 00001(ATCC® 11778; NCTC 10320)	50-100 CFU	18-24 h to 40-48 h/ 37 ± 1°C	Good growth, turquoise-blue colonies with precipitation halo (lecithinase activity)
Escherichia coli	WDCM 00013 (ATCC® 25922; NCTC 12241)	10^4-10^6 CFU	40-48 h/ 37 ± 1°C	Total inhibition
Bacillus subtilis	WDCM 00003 (ATCC® 6633; NCTC 10400)	10^3-10^4 CFU		White colonies without precipitation halo

Please refer to the actual batch related Certificate of Analysis (CoA).

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

WASTE DISPOSAL

Waste disposal must be carried out according to local and national legislation in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

Product	Format	Packaging	Ref.
Bacillus Cereus Agar (PEMBA)	Plate 90 mm	20 plates	10007

Significant changes from previous version:

Document	Release Date	Change Summary
10007_IFU-0	2022-10-27	Layout and content updated, version reset to revision 0.

This IFU document and the SDS are available from the online Support Center:
liofilchem.com/ifu-sds

References

1. EN ISO 11133:2014+Amd1:2018+Amd2:2020. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
2. 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
3. Holbrook R, and Anderson JM, (1980). An im-proved selective and diagnostic medium for the isolation of Bacillus cereus in foods. Can.J. Microbiol. 26, 753-759.
4. ICMSF (1978). Microorganisms in Foods. Toronto University Press. 274-275.
5. Mossel DAA, Koopman M J, Jongerius E (1967): Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15, 650-653.

Table of Symbols

LOT	Batch code
REF	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
Σ	Contains sufficient for <n> tests
	Consult Instructions For Use
	Do not reuse

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