Instructions for Use

REF - LS1051 **Bacillus cereus (PEMBA) Supplement**

Freeze-Dried Supplement

1. Intended Use

For in vitro diagnostic use. LS1051 Bacillus cereus (PEMBA) Supplement is an antibiotic supplement used to enhance the selective isolation of *Bacillus cereus* from foodstuffs and clinical specimens.

2. Composition* For one litre of prepared media

Ingredient	mg/L
Each vial contains:	
Polymyxin B	100000IU

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS1051 Bacillus cereus (PEMBA) Supplement is formulated to be used with E&O KM0186 Bacillus cereus medium and BM0140 Egg Yolk Emulsion to form Bacillus cereus Selective Agar (PEMBA).

Bacillus cereus Selective Agar (PEMBA) was developed by Holbrook and Anderson [(1)] for the isolation and enumeration of *B. cereus* in foods. It meets the requirements for a medium that is sufficiently selective to be able to detect small numbers of *B. cereus* cells and spores in the presence of large numbers of other food contaminants. The medium is also sufficiently diagnostic that colonies of *B. cereus* are readily identified and confirmed by microscopic examination. It also complies with ISO 21871 [(2)].

4. Principle

Bacillus cereus Selective Agar (PEMBA) is made selective by the inclusion of polymyxin B.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water. Close the vial and gently agitate to assist reconstitution. Avoid frothing and the resulting solution will be free from visible particulate matter.
Add the contents of the vial to the appropriate volume of sterile base medium, KM0186 Bacillus cereus medium and BM0140 Egg Yolk Emulsion, prepared according to the instructions provided and allowed to cool to 45°C- 50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	White Solid Pellet	Colourless liquid

7. Materials Provided

LS1051 Bacillus cereus (PEMBA) Supplement is supplied 10 x 5ml neutral clear crimped vial suitable for 500ml of media per box (lyophilised) (product code: LS1051-V001-500). Each vial is labelled with product name, product code, lot number and expiry date.

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms, sterile media (e.g., Bacillus cereus Selective Agar (PEMBA) made up from KM0186 Bacillus cereus medium and BM0140 Egg Yolk Emulsion)

9. Specimens

LS1051 Bacillus cereus (PEMBA) Supplement can be used with the following specimens when added to KM0186 Bacillus cereus medium and BM0140 Egg Yolk Emulsion to form Bacillus cereus Selective Agar (PEMBA):

- Food Industry: products for human consumption, animal feed and environmental samples
- Clinical: rectal swabs and stool specimens.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:10 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, media should be inoculated with 1ml of the specimen and incubate at 30 ± 1°C aerobically for 24 hours.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 30 ± 1°C aerobically for 24 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). If no initial colonies were seen at 24 hours, re-incubate the for a further 24 hours at 30 ± 1°C aerobically. Perform further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
B. cereus (NCTC 10320)	30 ± 1°C aerobically for 24-48 hours	Turquoise/blue colonies with zone of egg yolk precipitate of the same colour
B. subtilis (NCTC 10400)	30 ± 1°C aerobically for 24-48 hours	Yellow colonies
E. coli (NCTC 12241)	30 ± 1°C aerobically for 24-48 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium produced, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify LS1051 Bacillus cereus (PEMBA) Supplement performance, supplement samples were added to sterilised KM0186 Bacillus cereus medium and BM0140 Egg Yolk Emulsion and resulting plates tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated 30 ± 1°C aerobically for 24-48 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Bacillus cereus* (NCTC 10320) and *Bacillus subtilis* (NCTC 10400). Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10^4 - ≤1.5x10^5 cfu of the required non-target test organisms: Escherichia *coli* (NCTC 12241), which were inhibited and have a selectivity factor of ≥ 3. Therefore, it can be concluded that LS1051 Bacillus cereus (PEMBA) Supplement meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- It should be noted that some *Proteus* spp. and Gram-positive cocci may grow on this medium.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to LS1051 Bacillus cereus (PEMBA) Supplement Safety Data Sheet prior to use. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Do not use if supplement is not specified colour, has been stored inappropriately or the packaging has been damaged. Once reconstituted, any unused supplement should be discarded.

15. Storage conditions and Shelf life

LS1051 Bacillus cereus (PEMBA) Supplement must be stored at 2°-8°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements.

16. References

1. Holbrook, R. and Anderson, J. (1980) An improved selective and diagnostic medium for the isolation of Bacillus cereus in foods. Can. J. Microbiol. 26(7), pp.753-759.
2. International Organization for Standardization (2006) 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. Geneva: ISO.

Revision

001 21/03/17 - New document
002 01/08/19 - Format updated
003 05/10/23 - Updated to new standard

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

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place