REF AEB184001/10

184001 **D** – en- 2015/11 EN

POLYMYXIN B

For microbiological control only

Supplement for Mossel agar for the presumptive enumeration of cereus group *Bacillus* in food sample

SUMMARY AND EXPLANATION

Polymyxin B supplement is one of the supplements used for the preparation of Mossel agar used for the detection and enumeration of spores and vegetative cells of *Bacillus cereus* in foodstuffs.

PRINCIPLE

A presumptuous identification is carried out through the screening of typical colonies after incubation.

* B*. cereus* does not ferment mannitol. This characteristic helps to differentiate B*. cereus* from contaminating microorganisms which ferment mannitol, causing phenol red to turn yellow.

* *B. cereus* synthesize lecithinase, its action on the egg yolk lecithin produces insoluble breakdowns that accumulate around the colonies, forming a whitish precipitation.

Finally, Polymyxin B can be added to inhibit accompanying microflora when the tested sample is heavily contaminated.

CONTENT OF THE KIT

Lyophilised supplement		
REF AEB184001/10	Pack of 10 vials of	50.000 UI

COMPOSITION

Theoretical formula per unit

This medium can be adjusted and/or supplemented according to the performance criteria required:

Polymyxin B.....50.000 UI

MATERIAL AND REAGENTS REQUIRED BUT NOT PROVIDED

Material

- Bacteriology incubator
- Water baths
- Sterile or aseptic Petri plates

Reagents

- Mossel base (Ref. AEB151732 or Ref.AEB621736)
- Egg yolk emulsion 50% (Ref.AEB680102 or Ref. AEB680107)
- Blood agar (ex : Ref. AEB120650)
- Diluent (ex : Peptone salt Ref. AEB611498 / Ref. AEB111499)

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
  All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers from Occupationally Acquired Infections*; *Approved Guideline* – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents past the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use bottles which show signs of contamination.
- The medium must be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the bottles at 2-8 °C in their box until the expiry date.**

SPECIMENS

Follow the recommendations in the current standards to perform specimen collection and preparation.

PREPARATION

1. Let the bottles of Mossel base (ready to use or prepared from de dehydrated medium) come to room temperature, if necessary. Loosen the cap on the bottle of agar.
2. Place the bottle of agar in a water bath equipped with a security system set to approximately 50°C, increase the temperature to 100°C and leave the agar to melt (approximately 20 minutes).
3. Mix after screwing the cap back on (use protective gloves against thermal risks).
4. Leave the bottles at room temperature for at least 15 seconds before transferring them to a thermostatically controlled water bath set at approximately 47°C (+/-2°C).
5. Add 5 ml of sterile purified water to a Polymyxin B supplement. Add aseptically 1 ml to 100 ml of base. Homogenize.
6. Add aseptically 4 ml of egg yolk emulsion to 100 ml base. Homogenize.
7. Pour all the complete medium into Petri plates.

INSTRUCTIONS FOR USE

1. Allow the plates to come to room temperature. (If necessary dry them in an incubator).
2. Inoculate the specimen: Transfer 0.1 ml of sample, stock solution or decimal dilutions to the surface of the agar.
3. Carefully spread the inoculum as quickly as possible using a loop.
4. Cover the plates and dry for approximately 15 minutes at room temperature.
5. Incubate with the cover bottom side at 30°C +/- 1 °C. The cultures are generally examined after 24 and 48 hours of incubation.

READING AND INTERPRETATION

Bacillus cereus colonies are large flat, rough irregular and pink (Mannitol -) surrounded by an opaque halo due to the presence of the lecithinase. These colonies are flat rough and tend to spread.

LIMITATIONS OF THE METHOD

Other microorganisms such as *Staphylococcus aureus, Serratia marcescens* and *Proteus vulgaris* are known to use the egg yolk. Continue identification by looking at the bacterial morphology, testing dextrose fermentation, nitrate reduction and the production of acetylmethylcarbinol (VP).

Once opened a flask of egg yolk emulsion can be used until its expiry date if it was manipulated according to good laboratory practice (ie: Use in aseptic conditions and storage at 2 to 8°C).

QUALITY CONTROL

The supplement Polymyxin B has been designed and developed to meet the strictest quality requirements.

The results obtained using strains tested during controls for bacteriological activity are shown on the quality control certificate for each batch, available from our website (www.biomerieux.com).

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly. Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. Donovan K.O. 1958. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. **21**:100-103.

2. Mossel D.A.A., Koopman M.J. and Jongerius E. 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. **15**:650-653.

3. AFNOR NF EN ISO 7932 Juillet 2005. Microbiologie des aliments - Directives générales pour le dénombrement de *Bacillus cereus*. Méthode par comptage des colonies à 30°C.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use

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