Instructions for Use

REF - KM0084 **_Bacillus cereus_ Agar (MYP)**

Dehydrated Culture Media

1. Intended Use

KM0084 *Bacillus cereus* Agar (MYP) is a differential selective media intended for the enumeration of *Bacillus cereus* in food samples.

2. Composition*

Ingredient	g/L
Beef Extract	1.0
Peptone	10.0
Mannitol	10.0
Sodium Chloride	10.0
Phenol Red	0.025
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Bacillus cereus Agar (MYP) was originally developed by Mossel in 1967 as an effective way to enumerate *B. cereus* in food samples [1].

KM0084, when prepared as a final medium, is recommended by the International Organization for Standardization [2 3] and is tested in accordance with ISO 11133:2014 [4].

4. Principle

Bacillus cereus Agar (MYP) utilizes two reactions namely mannitol fermentation and lecithinase production to differentiate *B. cereus* from other related species. As *B. cereus* is mannitol negative the colonies are pink in colour due to the presence of the phenol red pH indicator. Lecithinase production (from the addition of egg yolk) is indicated by a white precipitate around the colonies. Beef extract and peptone supply the required carbon, nitrogen, and vitamins. Sodium chloride helps maintain the osmotic balance and phenol red is the pH indicator. Mannitol is a fermentable carbohydrate. The medium is made selective by the inclusion of polymyxin B sulphate (E&O LS0020).

5. Preparation Instructions

Suspend 46.0g/L of dehydrated culture medium in 1 litre of deionised/purified water and heat with frequent agitation and boil for one minute to completely dissolve powder.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C and aseptically add the appropriate volume of egg yolk emulsion (E&O BM0140) and *Bacillus cereus* MYP Selective Supplement (E&O LS0020).

Gently homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Buff fine powder	Peach firm gel
pH	N/A	7.2 ± 0.2

7. Materials Provided

KM0084 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0084-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0084-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0084-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

Ingredients for preparation of media supplied by E&O Laboratories *e.g.*, supplements E&O BM0140 Egg Yolk Emulsion and E&O LS0020 *Bacillus cereus* MYP Selective Supplement.

9. Specimens

KM0084 *Bacillus cereus* Agar (MYP) is suitable for the testing of the following specimens:

- Food Industry: products for human consumption, animal feed and environmental samples

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 aerobically at 30 ± 1°C aerobically for 44 ± 4 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). 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	Growth: Pink colonies with precipitation halo
B. subtilis (NCTC 10400)	30 ± 1°C aerobically for 24-48 hours	Growth: Yellow colonies with no precipitation halo
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, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0084 *Bacillus cereus* Agar (MYP) performance, dehydrated culture media samples were used to prepare *Bacillus cereus* Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 30 ± 1°C aerobically for 24-48 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Bacillus cereus* (NCTC 10320) and *Bacillus subtilis* (NCTC 10400) and no recovery of the non-target test organism: *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0084 *Bacillus cereus* Agar (MYP), 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. Results with atypical strains of *B. cereus* are quite variable, and further testing will be necessary to identify the isolates.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. 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.

Refer to KM0084 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Mossel, D., Koopman, M., and Jongerius, E. (1967) Enumeration of *Bacillus cereus* in foods. Appl. Microbiol., 15(3), pp.650-653.
2. International Organization for Standardization (2004) 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 degrees C. Geneva: ISO.
3. 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.
4. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.

Version History*

001 25/09/23 - New Document Created

*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		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place