

Instructions for Use

REF - PP0050

***Bacillus cereus* (MYP) Agar**

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use. PP0050 *Bacillus cereus* (MYP) Agar is a differential selective media intended for the enumeration of *Bacillus cereus* in food samples.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Beef extract	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	10.0
Phenol red	0.025
Agar	15.0
Egg yolk emulsion	100ml
Polymyxin B	100,000 IU

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Developed by Mossel in 1967 as an effective way to enumerate *B. cereus* in food samples ⁽¹⁾, this medium is formulated and tested to comply with the requirements of ISO7932 ⁽²⁾, ISO11133 ⁽³⁾ and ISO21871 ⁽⁴⁾.

4. Principle

Bacillus cereus (MYP) Agar 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.

5. Physical Characteristics

	Medium
Appearance and Colour	Peach firm gel
pH	7.2 ± 0.2

6. Materials Provided

PP0050 *Bacillus cereus* (MYP) Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0050-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

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

8. Specimens

PP0050 *Bacillus cereus* (MYP) Agar 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 for the conservation of *Bacillus spp.* Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. 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 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.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>B. cereus</i> (NCTC 10320)	Incubate aerobically at 30 ± 1°C for 44 ± 4 hours	Growth: Pink colonies with white halo
<i>B. subtilis</i> (NCTC 10400)	Incubate aerobically at 30 ± 1°C for 44 ± 4 hours	Growth: Yellow colonies
<i>E. coli</i> (NCTC 12241)	Incubate aerobically at 30 ± 1°C for 44 ± 4 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).

11. Performance

To fully verify PP0050 *Bacillus cereus* (Mannitol Egg Yolk Polymyxin – MYP) Agar performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10⁴-10⁵ cfu for the non-target organisms then incubated at 30 ± 1°C for 44 ± 4 hours. All samples showed expected recovery and 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 PP0050 *Bacillus cereus* (Mannitol Egg Yolk Polymyxin – MYP) Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. 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

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH 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 E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0050 in the original packaging between 2 and 8°C in the dark. 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. Do not use media if it is contaminated, discoloured, out of date or if cracks in the agar medium are present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

- Mossel, D., Koopman, M., and Jongerius, E. (1967) Enumeration of *Bacillus cereus* in foods. Appl. Microbiol., 15(3), pp.650-653.
- ISO (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.
- ISO (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva: ISO.
- ISO (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.

Version History*

001 14/04/23 - 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 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