

Bacillus Cereus MYP Agar (NCM0062)

Intended Use

Bacillus Cereus MYP Agar is used with supplements for the selective and differential enumeration of *Bacillus cereus* from foods. Conforms to ISO and FDA / BAM formulation in a laboratory setting. MYP Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Formulated to ISO 7932:2004 MYP was introduced by Mossel and his co-workers in 1967 for the enumeration of *Bacillus cereus* in foods and was shown to be the most effective for this purpose by Inal in 1972. *B. cereus* is differentiated from other members of the Bacillus group by two reactions: mannitol fermentation and lecithinase production. Mannitol fermentation on this medium produces a yellow color. *B. cereus* is mannitol negative and produces red colonies. The lecithinase production of *B. cereus* is indicated by a white precipitate around the colonies. Polymyxin is added to suppress coliforms but some *Proteus* spp. and Gram-positive cocci may grow.

Typical Formulation

Beef Extract	1.0 g
Peptone	10.0 g
D-Mannitol	10.0 g
Sodium Chloride	10.0 g
Phenol Red	0.025 g
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.

Supplements as required below

Egg Yolk Emulsion - 50% (7982 or X075), 50 mL* (**per ISO 7392/ISO 21871**) OR

Egg Yolk Emulsion - 50% (7982 or X075), 50 mL (**per FDA BAM**)

Polymyxin B Supplement (7997 or X193), 2 vials (10 mL)

*ISO 21871/ISO 7392 states that for usage of a commercial egg yolk emulsion, the concentration should be used according to the manufacturer's instructions. The amount of water used to prepare Bacillus Cereus MYP Agar is adjusted accordingly.

Precaution

Refer to SDS

Preparation per ISO 7392/ISO 21871

1. Suspend 46 g of the medium in 950 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and supplement per method below.
5. Aseptically add 50 mL of Egg Yolk Emulsion (X075 or 7982) and 2 vials of Polymyxin B Supplement (7997 or X193).

Preparation per FDA BAM

1. Suspend 46 g of the medium in 900 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45 - 50°C and supplement per method below.
5. Aseptically add 50 mL of Egg Yolk Emulsion (X075 or 7982) and 2 vials of Polymyxin B Supplement (7997 or X193).



Test Procedure

Refer to appropriate references for a complete discussion on the isolation and identification of *Bacillus cereus*.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is opalescent to opaque, yellow-orange to orange-pink.

Expected Cultural Response: Cultural response on *Bacillus Cereus* Agar Base at 30 ± 1°C after 21 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Bacillus cereus</i> ATCC® 11778	50-200	≥50% recovery; pink colonies with lecithinase halo
<i>Bacillus subtilis</i> ATCC® 6633	4 Quad Streak	Growth; Yellow colonies without lecithinase halo
<i>Escherichia coli</i> ATCC® 8739	>10 ⁴	Complete inhibition
<i>Escherichia coli</i> ATCC® 25922	>10 ⁴	Complete inhibition

The organisms listed are the minimum that should be used for quality control testing.

Results

Bacteria that ferment mannitol produce acid products and form colonies that are yellow. Bacteria that produce lecithinase hydrolyze lecithin and a zone of white precipitate forms around the colonies. *B. cereus* is typically mannitol-negative (pink colonies) and lecithinase positive (zone of precipitate around colonies).

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 7932:2004: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony-count technique at 30C.
2. Inal, T.: Vergleichende Untersuchungen über die Selektivmedien zum qualitativen und quantitativen Nachweis von *Vacillus cereus* in Lebensmitteln. Mitteilung I. : Fleischwritsch, 51: 1629-1632 (1971). IV. Mitteilung: Fleischwritsch, 52: 1160-1162 (1972).
3. Mossel, D.A.A., Koopman, M.J. and Jongerius, E. (1967). Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15: 650-653.
4. Thatcher, F.S., Clarke, D.S. (1978) Micro-organisms in foods. Volume 1 second edition. University of Toronto.
5. Donovan, K. O. 1598. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. 21:100-103.
6. Coliner, A. R. 1948. The action of *Bacillus cereus* and related species on the lecithin complex of egg yolk. J. Bacteriol. 55:777-785.

Technical Specification Sheet



7. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm
8. Downes, F. P. and K. Ito (eds). 2015. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
9. Andrews W. 1995. Microbial methods. In Official methods of analysis of AOAC International, 17th ed. AOAC International. Arlington, VA.
10. 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

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