

Product Instructions

Bacillus Cereus Mannitol Egg Yolk (MYP) Agar

Intended Use

Bacillus Cereus MYP Agar is used with supplements for the selective and differential enumeration of *Bacillus cereus* from foods. NCM0062 conforms to ISO and FDA BAM formulation. MYP Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A selective agar for the enumeration of presumptive *Bacillus cereus*. It is applicable to products intended for human consumption and the feeding of animals, and environmental samples in the area of primary production, food production and food handling, as described in ISO 7932:2004.

Formulated according 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, due to the indicator dye, phenol red changing color as acid is produced. *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. Beef extract and peptone provide the essential vitamins, minerals, amino acids, nitrogen and carbon, while sodium chloride maintains the osmotic balance. According to ISO 7932:2004, 0.1 mL–1.0 mL of a prepared test sample is transferred and spread over the agar surface. According to ISO 21871:2006 subculture is performed after enrichment in Tryptone Soya Polymyxin Broth.

Typical Formulation

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

Final pH: 7.2 ± 0.2 at 25°C

Formula is adjusted and/or supplemented as required to meet performance specifications.

Supplements as Required Below

Ordering Information	
NCM4017 or 700004877	Egg Yolk Emulsion – 50%* (per ISO 7932/ISO 21871 or per FDA BAM)
NCM4032 or 700004892	Polymyxin B Supplement

*ISO 21871/ISO 7932 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 safety data sheets.

Preparation per ISO 7932/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 NCM4017* or 700004877 Egg Yolk Emulsion and 2 vials of NCM4032-0.5* or 700004892 Polymyxin B Supplement each reconstituted using 5 mL of sterile deionized/RO water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

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 NCM4017* or 700004877 Egg Yolk Emulsion and 2 vials of NCM4032-0.5* or 700004892 Polymyxin B Supplement each reconstituted using 5 mL of sterile deionized/RO water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

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	
		Recovery	Reaction
<i>Bacillus cereus</i> ATCC® 11778	50–200	≥50%	Pink colonies w/ precipitation halo
<i>Bacillus subtilis</i> ATCC® 6633	4Q Streak	Good Growth	Yellow colonies w/o precipitation halo
<i>Escherichia coli</i> ATCC® 8739	>10 ⁴	Total inhibition	N/A
<i>Escherichia coli</i> ATCC® 25922	>10 ⁴	Total inhibition	N/A

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. : Fleischwirtsch, 51: 1629-1632 (1971). IV. Mitteilung: Fleischwirtsch, 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.
7. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.html.
8. Salfinger, Yvonne and Tortorello, Mary Lou (eds). 2015. Compendium of methods for the microbiological examination of foods, 4–5 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.

