

Bacillus Cereus Agar Base (PEMBA) SKU: 700003452, 700003453, 700003454, 700003455 NCM0165

Intended Use

Bacillus Cereus Agar Base is used with Polymyxin B and Egg Yolk Emulsion for the isolation and presumptive identification of *Bacillus cereus* and is not intended for use in the diagnosis of human disease.

Description

Holbrook and Anderson described a highly selective and diagnostic medium (PEMBA) for the isolation and determination of *Bacillus cereus* from food. The medium is formulated to detect small numbers of *B. cereus* in the presence of large number of contaminants. This medium differentiates *B. cereus* from other bacteria based on resistance to polymyxin, lack of mannitol fermentation, and presence of lecithinase. *B. cereus* can be found on vegetables, processed foods, and in nature. *B. cereus* causes gastrointestinal illness if the organism is allowed to proliferate. Outbreaks of foodborne illness have been associated with boiled and cooked rice, cooked meats, and cooked vegetables.

Typical Formulation

| | |
|----------------------------|----------|
| Enzymatic Digest of Casein | 1.0 g/L |
| Mannitol | 10.0 g/L |
| Sodium Chloride | 2.0 g/L |
| Magnesium Sulfate | 0.1 g/L |
| Disodium Phosphate | 2.5 g/L |
| Monopotassium Phosphate | 0.25 g/L |
| Bromothymol Blue | 0.12 g/L |
| Sodium Pyruvate | 10.0 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

NCM4017 Egg Yolk Emulsion - 50%*

NCM4032 Polymyxin B Supplement

*ISO 21871 states that for usage of a commercial egg yolk emulsion, the concentration should be used according to the manufacturer's instruction.

Precaution

Refer to SDS

Preparation

1. Suspend 41 grams of the medium in 965 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. Aseptically add 25 mL of NCM4017* Egg Yolk Emulsion 50% and 2 vials of
 5. 700004892* Polymyxin B reconstituted using 5 mL sterile deionized/RO water.

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



Technical Specification Sheet



Test Procedure

Refer to appropriate references, for example ISO 21871:2006 for the use of this medium for the isolation and identification of *Bacillus cereus*.

Quality Control Specifications

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

Prepared Appearance: Prepared medium is yellow to yellow-green and opaque.

Expected Cultural Response: Cultural response on *Bacillus Cereus* Agar Base at $37 \pm 1^\circ\text{C}$ after 18 - 48 hours incubation.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|--------------------------------------|------------------------|---------------------|---|
| | | Recovery | Reaction |
| <i>Bacillus cereus</i> ATCC® 13061 | $>10^3$ | Growth | Turquoise-blue colonies w/ lecithin ppt. (halo) |
| <i>Bacillus cereus</i> ATCC® 11778 | 50-200 | $> 70\%$ | Turquoise-blue colonies w/ lecithin ppt. (halo) |
| <i>Bacillus coagulans</i> ATCC® 7050 | $>10^3$ | Complete inhibition | N/A |
| <i>Bacillus subtilis</i> ATCC® 6633 | $>10^3$ | Growth | White colonies w/o ppt. |
| <i>Bacillus subtilis</i> ATCC® 9372 | $>10^3$ | Growth | White colonies w/o ppt. |
| <i>Escherichia coli</i> ATCC® 25922 | $> 10^5$ | Complete inhibition | N/A |
| <i>Escherichia coli</i> ATCC® 8739 | $> 10^5$ | Complete 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 (blue 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 sealed bottle containing the dehydrated medium at $2 - 30^\circ\text{C}$. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Holbrook and Anderson. 1980. Can. J. Microbiol. 26:753-759.
2. Donovan, K. O. 1598. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. 21:100-103.
3. Coliner, A. R. 1948. The action of *Bacillus cereus* and related species on the lecithin complex of egg yolk. J. Bacteriol. 55:777- 785.
4. Harmon, S. M., J. M. Goepfert, and R. W. Bennett. 2015. *Bacillus cereus*, Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.



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Technical Specification Sheet



5. 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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