

## Bacillus Cereus Agar Base (PEMBA) (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 may be adjusted and/or supplemented as required to meet performance specifications.

### Supplements/Liter

Egg Yolk Emulsion - 50% (7982 or X075), 25 mL\*

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

\*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 g 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 Egg Yolk Emulsion (7982 or X075) and 10 mL of Polymyxin B (7997 or X193, 2 x 5mL vials).

### 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.



# Technical Specification Sheet



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

**Expected Cultural Response:** Cultural response on Bacillus Cereus Agar Base at 37 ± 1°C after 18 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Reaction
<i>Bacillus cereus</i> ATCC® 13061	>10 <sup>3</sup>	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 <sup>3</sup>	Complete inhibition	N/A
<i>Bacillus subtilis</i> ATCC® 6633	>10 <sup>3</sup>	Growth	White colonies w/o ppt.
<i>Bacillus subtilis</i> ATCC® 9372	>10 <sup>3</sup>	Growth	White colonies w/o ppt.
<i>Escherichia coli</i> ATCC® 25922	> 10 <sup>5</sup>	Complete inhibition	N/A
<i>Escherichia coli</i> ATCC® 8739	> 10 <sup>5</sup>	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°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. 1958. 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, 4<sup>th</sup> ed. American Public Health Association, Washington, D.C.
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

