

## BACILLUS CEREUS AGAR BASE (7442)

### Intended Use

**Bacillus Cereus Agar Base** is used with Polymyxin B and Egg Yolk Emulsion for the isolation and presumptive identification of *Bacillus cereus*.

### Product Summary and Explanation

Holbrook and Anderson described a highly selective and diagnostic medium (PEMBA) for the isolation and determination of *Bacillus cereus* from food.<sup>1</sup> 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.<sup>2,3</sup> *B. cereus* can be found on vegetables, processed foods, and in nature.<sup>4</sup> *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.<sup>5</sup>

### Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein in Bacillus Cereus Agar Base. Sodium Chloride maintains the osmotic environment. Mannitol is the carbohydrate, and fermentation is detected by the pH indicator Bromthymol Blue. Magnesium Sulfate provides divalent cations and sulfate. The Phosphates are buffering agents in the medium. Sodium Pyruvate enhances growth and lecithinase production. Agar is the solidifying agent. Supplementing with Egg Yolk Emulsion provides lecithin, and Polymyxin B inhibits growth of most other bacteria. In the event of a high mold count, Cycloheximide (40 mg/L) can be added.

### Formula / Liter

Enzymatic Digest of Casein .....	1 g
Mannitol .....	10 g
Sodium Chloride .....	2 g
Magnesium Sulfate .....	0.1 g
Disodium Phosphate.....	2.5 g
Monopotassium Phosphate .....	0.25 g
Bromthymol Blue.....	0.10 g
Sodium Pyruvate.....	10 g
Agar .....	15 g

Final pH: 7.2 ± 0.2 at 25°C

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

### Supplements

Egg Yolk Emulsion, 100 mL (7982)
Polymyxin B (50,000 IU), 5 mL (7997)

### Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 41 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. Aseptically add 50 mL of Egg Yolk Emulsion (# 7982) and 10 mL of Polymyxin B (# 7997, 2 x 5mL vials).

### Quality Control Specifications

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

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

**Expected Cultural Response:** Cultural response on Bacillus Cereus Agar Base at 30°C after 18 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Bacillus cereus</i> ATCC® 13061	1000	Fair to good	Blue colonies w/ lecithin ppt. (halo)
<i>Bacillus subtilis</i> ATCC® 9372	1000	Fair to good	Cream to light yellow colonies w/o ppt.
<i>Bacillus coagulans</i> ATCC® 7050	1000	Complete inhibition	N/A

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

### Test Procedure

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

### 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).

### 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.

### Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitation of the Procedure

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

### Packaging

<b>Bacillus Cereus Agar Base</b>	<b>Code No.</b>	<b>7442A</b>	<b>500 g</b>
		<b>7442B</b>	<b>2 kg</b>
		<b>7442C</b>	<b>10 kg</b>
<b>Egg Yolk Emulsion</b>		<b>7982</b>	<b>100 mL</b>
	<b>Polymyxin B (50,000 IU)</b>	<b>7997</b>	<b>5 mL</b>

### 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. **Jeffery, E. J. and S. M. Harmon.** 1995. *Bacillus cereus*, p. 14.01-14.08. In Bacteriological analytical manual, 8<sup>th</sup> ed. AOAC International, Gaithersburg, MD.
5. **Harmon, S. M., J. M. Goepfert, and R. W. Bennett.** 1992. *Bacillus cereus*, p. 593-604. In C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.

### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.