BACILLUS CEREUS AGAR BASE (7442)

Intended Use

Bacillus Cereus Agar Base is used with polymyxin B and egg yolk suspension 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.[1] 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.[2,3] *B. cereus* can be found on vegetables, processed foods, and in nature.[4] *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.[5]

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 suspension 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

Sterile Egg Yolk Suspension, 50 mL

Polymyxin B (100,000 units), 2 mL
(filtered sterilized aqueous)

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 and aseptically add 50 mL of a sterile egg yolk suspension and 2 mL of a filtered sterilized aqueous solution of polymyxin B (100,000 units).

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	Response	Reactions
Bacillus cereus ATCC® 13061	growth	blue colonies w/ halo
Bacillus subtilis ATCC® 9372	partial to complete inhibition	---

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

Bacillus Cereus Agar Base	**Code No.**	**7442A**	**500 g**
		7442B	**2 kg**
		7442C	**10 kg**

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. **Jeffery, E. J. and S. M. Harmon.** 1995. *Bacillus cereus*, p. 14.01-14.08. *In* Bacteriological analytical manual, 8th 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, 3rd 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.

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