IFU

Instructions for Use

BACILLUS CEREUS SELECTIVE AGAR

Cat. no. G156	Bacillus cereus Selective Agar, 15x100mm plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Bacillus cereus Selective Agar is a selective medium for the isolation and differentiation of *Bacillus cereus* in foods.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

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 a large number of other contaminants. Bacillus cereus Selective Agar has the ability to differentiate *B. cereus* from other species of *Bacillus* based on its resistance to polymyxin, lack of mannitol fermentation, and the presence of lecithinase. *B. cereus* can be present in a variety of produce and processed foods and the organism can cause gastointestinal illness if allowed to proliferate. Past outbreaks have been associated with the ingestion of rice, cooked meats, and cooked produce contaminated with *B. cereus.*

Hardy Diagnostics Bacillus cereus Selective Agar contains nitrogen, carbon, and meat peptone, which provide essential nutrients. Sodium chloride maintains osmotic equilibrium. The addition of sodium pyruvate enhances sporulation and egg yolk precipitation. Magnesium sulfate provides divalent cations which act as special growth factors for *Bacillus* spp. Mannitol fermentation is detected by the pH indicator bromothymol blue, resulting in *Bacillus cereus* colonies having a distinctive turquoise blue color. Egg yolk suspension is added as a source of lecithin to demonstrate the lecithinase reaction. The addition of polymyxin B inhibits gram-negative bacteria.

Typical colonies of *B. cereus* are crenated, about 5mm in diameter, and have a distinctive turquoise to peacock blue color surrounded by a zone of precipitate of the same color. These features distinguish *B. cereus* from other *Bacillus* species, except *B. thuringiensis*. Other egg yolk-reacting organisms which can grow on this medium, including *Staphylococcus aureus*, *Serratia marcescens* and *Proteus vulgaris*, are distinguished from *B. cereus* by colony morphology and color. These organisms produce an egg yolk clearing reaction in contrast to the egg yolk precipitate produced by *B. cereus.*

Microscopic examination for the presence of lipid globules in the vegetative cells is recommended as a rapid and confirmatory test for *B. cereus* and replaces the need for biochemical testing. Holbrook and Anderson have confirmed that only *B. cereus* in this genus is capable of possessing lipid globules in its vegetative cells when grown on the selective medium.[1] One further advantage of this test is that strains of *B. cereus* that react only weakly or not at all with egg yolk can be detected and confirmed.

FORMULA

Ingredients per 900ml of deionized water:*

Mannitol	10.0gm
Sodium Pyruvate	10.0gm
Disodium Phosphate	2.5gm
Sodium Chloride	2.0gm
Peptic Digest of Animal Tissue	1.0gm
Dipotassium Phosphate	0.25gm
Bromothymol Blue	0.12gm
Magnesium Sulfate	0.1gm
Sterile Egg Yolk Suspension	100ml
Polymyxin B	100,000IU
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample collection: Consult listed references for appropriate sampling plans.[2-5]

1.Samples should be processed in a manner suitable for the source material: liquid, solid, semi-solid, or frozen.

2. Prepare appropriate decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

3. Aseptically inoculate the agar surface with 0.1ml of a well mixed diluted sample.

4. Using a sterile spreader device, spread the inoculum evenly over the surface of the agar to achieve isolated colonies.

INTERPRETATION OF RESULTS

Typical colonies of *B. cereus* are crenated, about 5mm in diameter and have a distinctive turquoise to peacock blue color surrounded by a zone of precipitate of the same color. These features distinguish *B. cereus* from other *Bacillus* species, except *B. thuringiensis*. Other egg yolk-reacting organisms which can grow on the medium, including *Staphylococcus aureus*, *Serratia marcescens* and *Proteus vulgaris*, are distinguished from *B. cereus* by colony morphology and color. These organisms produce an egg yolk clearing reaction in contrast to an egg yolk precipitate produced by *B. cereus*.

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LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

On this medium, *B. cereus* is indistinguishable from *B. thuringiensis*. *B. thuringiensis* can be distinguished from *B. cereus* using molecular methods or the protein toxin crystal stain.

Occasional strains of *B. cereus* shows weak or negative egg yolk reactions.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacillus cereus ATCC® 13061**	A	24-48hr	35°C	Aerobic	Growth; blue colonies with opaque halo

Bacillus spizizenii ATCC® 6633**	A	24-48hr	35°C	Aerobic	Partial to complete inhibition. Media remains yellow.
Pseudomonas aeruginosa ATCC® 27853**	B	24-48hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

** Recommended QC strains for User Quality Control.

PHYSICAL APPEARANCE

Bacillus cereus Selective Agar should appear translucent, and yellow in color.

REFERENCES

1. Can. 1980. *J. Microbiol.*; 26:753-7590.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

3. Association of Official Analytical Chemists. *Official Methods of Analysis*, AOAC, Washington, D.C.

4. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.