

Instructions for Use

CRITERION™ BACILLUS CEREUS AGAR BASE

Cat. no. C5060	CRITERION™ Bacillus Cereus Agar Base	82gm
Cat. no. C5061	CRITERION™ Bacillus Cereus Agar Base	500gm
Cat. no. C5062	CRITERION™ Bacillus Cereus Agar Base	2kg
Cat. no. C5063	CRITERION™ Bacillus Cereus Agar Base	10kg
Cat. no. C5064	CRITERION™ Bacillus Cereus Agar Base	50kg

INTENDED USE

IFU

Hardy Diagnostics CRITERION[™] Bacillus Cereus Agar Base is a selective agar medium used for the isolation and determination of *Bacillus cereus*.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

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 contaminants. Colonies of *B. cereus* growing on this medium can readily be identified by microscopic examination. The addition of sodium pyruvate enhances egg yolk precipitation and sporulation. Mannitol-fermentation is detected by the pH indicator bromothymol blue. Selective activity is produced by the addition of polymyxin B at a concentration of 100,000 units per liter.^(2,3) In the event of high mold content, cycloheximide (40mg/L) can be added.

FORMULA

Gram weight per liter:	41.0gm/L
Mannitol	10.0gm
Sodium Pyruvate	10.0gm
Disodium Phosphate	2.5gm
Sodium Chloride	2.0gm
Peptic Digest of Animal Tissue	1.0gm
Dipotassium Phosphate	250.0mg
Bromothymol Blue	120.0mg

Magnesium Sulfate	100.0mg
Agar	15.0gm

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original off-white.

Store the prepared culture media at 2-8°C.

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 "<u>Guidelines for Isolation</u> <u>Precautions</u>" 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 41.0gm of the dehydrated culture media in 950ml of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Distribute and sterilize in the autoclave at 121°C for 15 minutes.

4. Cool to 45-50°C and aseptically add enrichments (50ml of a sterile egg yolk suspension and 2ml of a filtered solution of polymyxin B (100,000units). If required, cycloheximide may be added at a concentration of 40mg/L.

5. Mix thoroughly and dispense.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. G156.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

On this medium, B. cereus is indistinguishable from B. thuringiensis.

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 autoclaves, incinerators, and incubators, etc., 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	Results
Bacillus cereus ATCC [®] 13061	А	24-48hr	35°C	Aerobic	Growth; blue colonies with blue precipitate halos
Bacillus spizizenii ATCC [®] 6633	В	24-48hr	35°C	Aerobic	Partial to complete inhibition; media remains yellow
Pseudomonas aeruginosa ATCC [®] 27853	В	24-48hr	35°C	Aerobic	Inhibited

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

USER QUALITY CONTROL

Users of dehydrated 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 <u>Certificate of Analysis</u> website. In addition, refer to the following document "<u>Finished Product</u> <u>Quality Control Procedures</u>," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION[™] Bacillus Cereus Agar Base powder should appear homogeneous, free-flowing, and off-white in color.

The prepared media should appear clear to a trace hazy, and blue in color.

REFERENCES

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

2. Donovan, K.O. 1958. J. Appl. Bacteriol.; 21(1):100-103.

3. Mossel, D.A.A., et al. 1967. J. Appl. Bacteriol.; 15(3):650-653.

4. Vanderzant, C. and D.F. Splittstoesser, (ed.). *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10115[B]



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Distribution Centers: California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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