PRODUCT SPECIFICATION SHEET

Bacillus Cereus Agar Base (DM033)

Intended Use
Bacillus Cereus Agar (DM033) is used for selective isolation detection and enumeration of Bacillus cereus.

Product Summary and Explanation
Bacillus Cereus Agar is based on the highly specific diagnostic and selective PEMBA medium, developed by Holbrook and Anderson[1]for the isolation and enumeration of *Bacillus cereus* in foods. It meets the requirements for a medium that is sufficiently selective to be able to detect small numbers of *B. cereus* cells and spores in the presence of large numbers of other food contaminants. The role of *B. cereus* in food poisoning, particularly from the consumption of contaminated rice, is now well documented[2,3,4] The organism has also been implicated in eye infections[5,6] and a wide range of other conditions including abscess formation, meningitis, septicaemia and wound infection. *B. cereus* is recognised as a significant pathogen in post-operative and post-traumatic wounds of orthopaedic patients.[7] *Bacillus cereus* is a known cause of disease mastitis, especially in ewes and heifers among the veterinarians.[8]
The primary diagnostic features of the medium are the colonial appearance, precipitation of hydrolysed lecithin and the failure of *B. cereus* to utilise mannitol. The typical colonies of *Bacillus cereus* are crenated, about 5 mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour. The bacteria do not ferment mannitol and thus there is no change in colour of the indicator dye around the colonies.
Addition of polymyxin-B sulphate[9,10] at a final concentration of 100 units per ml of medium is sufficient to make the medium selective for the isolation of *Bacillus cereus* . It suppresses the growth of accompanying bacterial flora. If moulds are suspected in the inoculum, 40 mcg per ml filter-sterilized cycloheximide may be incorporated to suppress the mould contamination. Some strains of *Bacillus cereus* have very weak egg yolk reaction. Moreover, on this medium *Bacillus cereus* is indistinguishable from *Bacillus thuringiensis*.

Principles of the Procedure
The nitrogen, vitamin, and carbon sources are provided by Peptic Digest of animal tissue in Bacillus Cereus Agar Base. Sodium Chloride maintains the osmotic environment. Mannitol is the carbohydrate, and fermentation is detected by the pH indicator Bromothymol 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

Ingredients	Gms / Liter
Peptic digest of animal tissue	1.00
Mannitol	10.00
Sodium chloride	2.00
Magnesium sulphate	0.10
Disodium phosphate	2.50
Monopotassium phosphate	0.25
Sodium pyruvate	10.00
Bromo thymol blue	0.12
Agar	15.00

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Final pH: 7.2 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications

Precautions

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

Directions

1. Suspend 20.5 grams in 475 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes/ validated cycle.
4. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective Supplement (MS002) and 25 ml of sterile Egg Yolk Emulsion (MS038).
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to greenish yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Green coloured clear to slightly opalescent gel. After addition of egg yolk emulsion : Yellowish green coloured opaque gel forms in Petri plates
Reaction of 4.1% Solution (basal medium)	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Polymyxin B Selective Supplement (MS002) and Egg Yolk Emulsion (MS038) after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Colour of colony	Egg Yolk Reaction
1.	*Bacillus cereus ATCC 10876*	50 -100	Good-luxuriant	>=50%	blue	positive, precipitation
2.	*Escherichia coli ATCC25922*	>=10^3	Inhibited	0%	--	--
3.	*Proteus vulgaris ATCC13315*	50-100	Good-luxuriant	>=50%	green	negative
4.	*Serratia marcescens ATCC 8100*	50-100	Good-luxuriant	>=50%	yellow-light pink (pigment production is enhanced by incubation at 25-30°C)	negative
5.	*Staphylococcus aureus ATCC 25923*	50-100	Good-luxuriant	>=50%	yellow	positive, clearing

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

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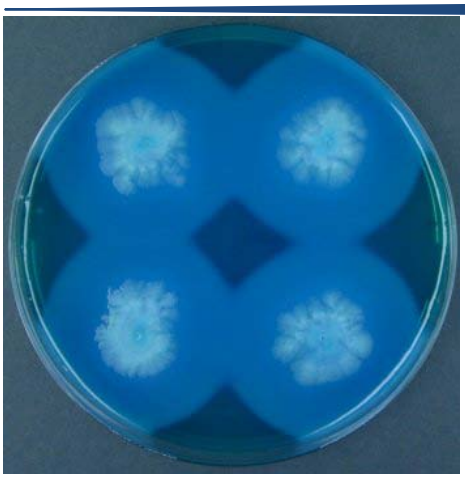
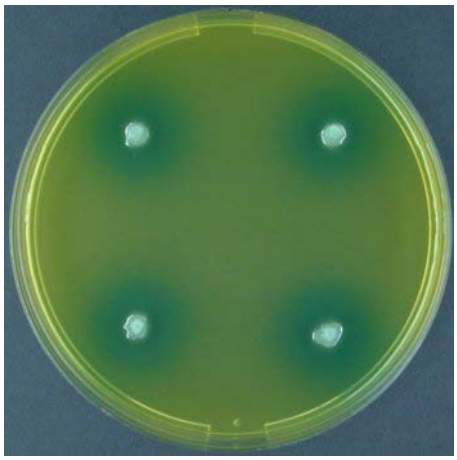


Bacillus cereus: Typical blue colonies surrounded by an egg yolk precipitate



Bacillus subtilis: Green colonies with no egg yolk precipitate

Test Procedure

For the isolation and enumeration of *Bacillus cereus* in foodstuffs the following method is recommended.

1. Homogenise 10g of the food sample for 30 seconds in 90ml of 0.1% Peptone Water (DM192) using a Stomacher.
2. Dried foods should first be rehydrated by soaking 20g in 90ml of Tryptone salt solution (Tryptone 0.3% and sodium chloride 0.8%, pH 7.3) for 50 minutes at room temperature.
3. Add a further 90ml of 0.1% peptone water to give a final dilution of 10^{-1}. Homogenise for 30 seconds using the Stomacher.
4. Further dilutions of the homogenate should be made in 0.1% peptone water.
5. Inoculate 0.1ml amounts of the 10-1 and higher dilutions on to the surface of the medium.
6. Incubate at 37°C under aerobic conditions for 24-48 hours.
7. Possible growth of contaminants is greatly reduced by incubation for 24 hours.
8. Examine for typical colonies of B. cereus. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample.
9. Confirmatory tests should be carried out before interpretation.

The medium may also be used for detecting B. cereus in milk.

1. When necessary, decimal dilutions of the samples should be made in 0.1% peptone water.
2. Undiluted and diluted samples are inoculated directly on to plates of agar and incubated.
3. An incubation temperature of 38°C for 18 hours is recommended as optimal for promoting the growth of B. cereus relative to that of other organisms.[9]

For examining clinical specimens plates may be inoculated in the usual way.

Results

1. Bacteria that ferment mannitol produce acid products and form colonies that are yellow.
2. Bacteria that produce lecithinase hydrolyze lecithin and a zone of white precipitate forms around the colonies.
3. *B. cereus* is typically mannitol-negative (blue colonies) and lecithinase positive (zone of precipitate around colonies).

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Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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

Limitations of the Procedure
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium

Packaging
Product Name : Bacillus Cereus Agar Base
Product Code : DM033
Available Pack sizes : 100gm / 500gm

References

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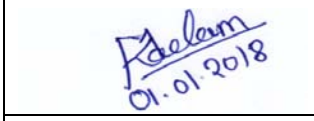		
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