Bacillus Cereus Agar Base

Intended Use
Bacillus Cereus Agar Base with added supplements is used as a selective medium for the isolation and enumeration of *Bacillus cereus* from food samples.

Summary
Bacillus Cereus Agar Base, with selective supplements, is based on the highly specific diagnostic and selective PEMBA medium, developed by Holbrook and Anderson for the isolation and enumeration of *B. cereus,* which is a known cause of the disease mastitis, especially in ewes and heifers. This media supports the growth of even a small number of *B. cereus* cells and spores in the presence of other food contaminants. Colonies of *B. cereus* can be readily identified and confirmed by microscopic examination.

Principle
Peptic digest of animal tissue and sodium pyruvate improve egg yolk precipitation and enhance sporulation. Bromothymol blue acts as pH indicator to detect mannitol fermentation. It suppresses the growth of accompanying bacterial flora. In case mould contamination is suspected in the inoculum, 40 mcg per mL filter-sterilized cycloheximide may be added to suppress the growth of mould. The primary diagnostic features of the medium are the colonial appearance, precipitation of hydrolyzed lecithin and the failure of *B. cereus* to utilize mannitol. The typical colonies of *B. cereus* are crenated, 5 mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour. These features distinguish *B. cerues* from other *Bacillus* species except *B. thuringiensis.* Other egg yolk-precipitating organisms, which can grow on the medium, include *S. aureus*, *S. marcescens* and *P. vulgaris*, which are distinguished from *B. cereus* by colony form and colour. These organisms also produce an egg yolk-clearing reaction in contrast to the egg yolk precipitate formed by *B. cereus*.

Formula*

Ingredients	**g/L**
Peptic Digest of Animal Tissue	1.0
Mannitol	10.0
Sodium Pyruvate	10.0
Sodium Chloride	2.0
Magnesium Sulphate	0.1
Monopotassium Phosphate	0.25
Disodium Phosphate	2.5
Bromothymol Blue	0.12
Agar	15.0
Final pH (at 25°C)	7.2 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Food and dairy samples, Veterinary samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 20.50 g of the powder in 475 mL purified / distilled water.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 45°C-50°C and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective Supplement (204160710005) and 25 mL of sterile Egg Yolk Emulsion (204050370100).
5. Mix well and pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Green to greenish yellow coloured, homogeneous, free-flowing powder.
Prepared Appearance: Basal medium yields - Green coloured, clear to slightly opalescent gel. With addition of 5% Egg Yolk Emulsion - yellowish green coloured, opaque gel forms in petridishes.
Cultural Response: Cultural characteristics observed with added Polymyxin B Selective Supplement (204160710005) and Egg Yolk Emulsion (204050370100) after an incubation of 24-48 hours at 35°C-37°C.

Organisms (ATCC)	Growth	Colour of colony	Egg Yolk Reaction
Bacillus cereus (10876)	Good	Blue	Precipitation
Escherichia coli (8739)	Inhibited	-	-
Proteus hauseri (13315)	Good	Green	-
Staphylococcus aureus subsp. *aureus* (25923)	Good	Yellow	Clearing

Interpretation of Results

1. Typical colonies of *B. cereus* are crenated, about 5 mm in diameter with a distinctive turquoise to peacock blue surrounded by a good egg yolk precipitate of the same colour.
2. The spores stain pale green to mid green, are paracentral or central in position and do not swell the sporangium.
3. Lipid globules and the vegetative cytoplasm are both red.
4. Only *B. cereus* are capable of possessing lipid globules in their vegetative cells when grown on selective medium and the presence of lipid globules is recommended as a rapid and confirmatory test for *B. cerues*.

For quantitative test

1. Leave the plated for a further 24 hours at room temperature in order to detect all the *Bacillus cereus* colonies.
2. Report the results as the number of *B. cereus* colonies per gram weight of the food sample.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Identify *B. cereus* by colony form, colour, egg yolk hydrolysis and confirm with cell and spore morphology.
2. Occasional strains of *B. cereus* show weak or negative egg yolk reactions.
3. Confirm the presumptive identification of *B. cereus* by the rapid confirmatory staining procedure.
4. On this medium *B. cereus* is indistinguishable from *B. thuringiensis*. Other organisms like *S. aureus*, *P. vulgaris* and *S. marcescens* may also grow on this medium.

Warranty

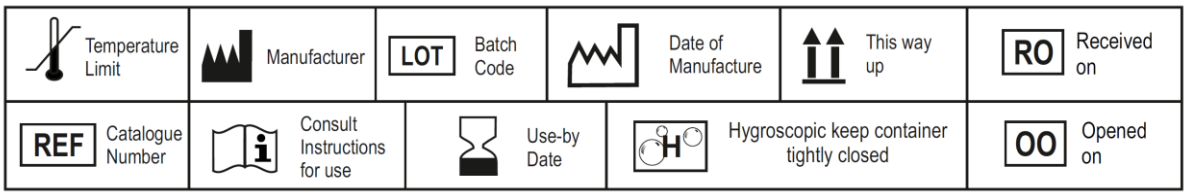
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Holbrook R and Anderson J; 1980, Can. J. Microbiology; 26(7): 753.
2. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020040100	Dehydrated Culture Media	100 g
201020040500	Dehydrated Culture Media	500 g
201020042500	Dehydrated Culture Media	2.5 k



Revision: 0825/VER-03

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.