

Bacillus Cereus Selective Agar Base (PEMBA) ISO

Cat. 1125

For the enumeration and isolation of *Bacillus cereus* in food.

Practical information

Applications	Categories
Selective enumeration	<i>Bacillus cereus</i>

Industry: Food

Regulations: ISO 11133 / ISO 21871

Principles and uses

Bacillus Cereus Selective Agar Base (PEMBA) has been adapted to meet the nutritional needs of *Bacillus cereus*, and was proposed for the enumeration, detection and isolation of *Bacillus cereus* in food. This bacterium is resistant to certain concentrations of polymyxin, which inhibits the accompanying flora, and is effective mainly against Gram-negative organisms. This medium is recommended by ISO 21871 for the determination of low number of *Bacillus cereus*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sodium pyruvate reduces the colony size of the organisms. Bromothymol blue acts as pH indicator to detect mannitol fermentation. Mannitol is the fermentable carbohydrate providing carbon and energy, *Bacillus cereus* is mannitol-negative. The Mannitol content allows the identification of the accompanying mannitol positive flora. Sodium chloride maintains the osmotic balance of the medium. Bacteriological agar is the solidifying agent.

Bacillus cereus produces lecithinases. The insoluble degradation products from the lecithin of egg yolk accumulate around the *Bacillus cereus* colonies, forming a white precipitate. The colonies of *Bacillus cereus* will appear blue colonies surrounded by a zone of opacity in the medium.

Formula in g/L

Enzymatic digest of casein	1	Bromthymol blue	0,12
Bacteriological agar	14	D-mannitol	10
Magnesium sulfate heptahydrated	0,1	Potassium dihydrogen phosphate	0,25
Sodium chloride	2	Sodium hydrogen phosphate	2,5
Sodium pyruvate	10		

Preparation

Suspend 40 grams of the medium in 950 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50 °C and aseptically add 50 ml of Egg Yolk Emulsion (Cat. 5152) and, if desired, aseptically add 2 vials of *Bacillus Cereus* Supplement (Cat. 6021). Homogenize gently and dispense into appropriate containers.

Instructions for use

For enumerate low numbers of *Bacillus cereus* according to ISO 21871:

- Enrichment in selective liquid medium TSPB:

3 tubes with 10 ml of medium at double concentration.

3 tubes with 9 ml of the medium at simple concentration.

- Inoculate the double concentration tubes with 10 ml of the initial suspension of the sample to be analyzed, and inoculate the single concentration tubes with 1 ml of the initial suspension or subsequent dilutions.

- Inoculate at 30 °C for 48±4 hours.

- Shake the tubes gently and inoculate the Selective Agar for *Bacillus Cereus* (PEMBA) with a sowing handle (10 µl).

- Incubate the plates in inverted position 37 °C for 18-24 hours. If the colonies have not been identified correctly, continue with the incubation for 24 hours more.

- Examine the plates to detect the presence of colonies.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Bluish-cream	Greyish-blue	7,2±0,2

Microbiological test

According to ISO 11133:

Incubation conditions: Productivity (21±3 - 44±4 h/37±1 °C) / Specificity, Selectivity (44±4 h/37±1 °C).

Inoculation conditions: Productivity qualitative (10³ - 10⁴ CFU) / Selectivity (10⁴-10⁶ CFU) / Specificity(10³-10⁴ CFU).

Reference media: TSA

Microorganisms	Specification	Characteristic reaction
Bacillus cereus ATCC 11778	Good growth (2)	Turquoise colonies with precipitation halo
Escherichia coli ATCC 25922	Total inhibition (0)	
Bacillus subtilis ATCC 6633	Good growth	White colonies w/o precipitation halo

Storage

Temp. Min.:2 °C

Temp. Max.:25 °C

Bibliography

Donovan, K.O.: A Selective Medium for Bacillus cereus in Milk, J. appl. Bact., 21; 100:103 (1958)

Mossel. D.A.A. Koopman, M.J. a Jongerius, E.: Enumeration of Bacillus cereus in Foods. Appl. Microbiol., 1 5; 650:653 (1967)

ISO 21871 Horizontal method for the determination of low numbers of presumptive Bacillus cereus.