

BACILLUS CEREUS SELECTIVE AGAR BASE (MYP) ISO 7932

CAT N°: 1343

For the presumptive enumeration of *Bacillus cereus*

FORMULA IN g/l

Enzymatic Digest of Casein	10.00	Beef Extract	1.00
Sodium Chloride	10.00	Phenol Red	0.025
D-Mannitol	10.00	Bacteriological Agar	15.00

Final pH 7.2 ± 0.2 at 25°C

PREPARATION

Suspend 46 grams of the medium in 900 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 100 ml of Egg Yolk Emulsion (Cat. 5152) and aseptically add 2 vials of Bacillus Cereus Supplement (Cat. 6021) reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into appropriate containers. The prepared medium should be stored at 8-15°C. The color is red- orange.

The dehydrated medium should be homogeneous, free-flowing and pink-cream in color. If there are any physical changes, discard the medium.

Bacillus Cereus Supplement (Cat. 6021)
(1 vial for 500 ml of the medium)

Polymixin B 50.000 IU

USES

BACILLUS CEREUS AGAR BASE is a medium recommended by the ISO normative 7932 for the presumptive enumeration of *Bacillus cereus* with the count-plate technique at 30°C.

When, for certain products, it is desirable to estimate low numbers of *B. cereus*, the limits of detection may be raised by a factor of 10 by examining 1.0 ml of the test sample if the initial product is liquid, or 1.0 ml of the initial suspension for the other products. Distribute the 1 ml of inoculum either on the surface of a large Petri dish (140 mm) or over the surface of three small dishes (90 mm) using a sterile spreader (6.8). In both cases, prepare duplicates by using two large plates or six small plates.

Beef Extract and Enzymatic Digest of Casein provide nitrogen, vitamins, minerals and amino acids essential for growth. 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, which are characterized by a yellow color. Phenol red is the pH indicator. Bacteriological agar is the solidifying agent.

Bacillus cereus is resistant to certain concentrations of Polymyxin, which inhibits the accompanying flora, and is effective mainly against gram-negative organisms.

Bacillus cereus produces lecithinases. The insoluble degradation products of the lecithin of egg yolk accumulate around the *Bacillus cereus* colonies, forming a white precipitate. Inoculated plates should be incubated for 18 hours to 24 hours at 30°C. If colonies are not clearly visible, incubate the plates for an additional 24 hours before counting. The presumptive colonies are large, pink (indicating that mannitol fermentation has not occurred) and generally surrounded by a zone of precipitation (indicating the production of lecithinase).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with supplement and egg yolk added, from type cultures after incubation at a temperature of $30 \pm 2^\circ\text{C}$ and observed after 18-24 hours.

Microorganisms	Growth	Colony color	Precipitation
<i>Bacillus cereus</i> ATCC 11778	Good	Red	+
* <i>Bacillus subtilis</i> ATCC 6633	Good	Yellow	-
* <i>Proteus mirabilis</i> ATCC 29906	Inhibited	Colorless	-
<i>Staphylococcus aureus</i> ATCC 6538	Inhibited	Yellow	+

*Incubate at 37°C for 48 hours

According ISO 11133 Productivity (24-48 h/ 30°C) and Specificity, Selectivity (48h 37°C)

Microorganisms	Inoculum (cfu/ml)	Productivity Quantitative	Selectivity Qualitative	Specificity Qualitative
<i>Bacillus cereus</i> ATCC 11778	10^2	$pr \geq 0.9$		Pink with precipitation halo
<i>Bacillus subtilis</i> ATCC 6633	10^2			yellow colonies
<i>Escherichia coli</i> ATCC 25922	$10^4/10^6$		Inhibited	

Reference Media Productivity: TSA

BIBLIOGRAPHY

Internacional Standard ISO 7932 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive *Bacillus cereus* — Colony-count technique at 30°C .

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

STORAGE

Once opened keep powdered medium closed to avoid hydration.

