Technical Data Sheet

GranuCult®
PEMBA (Polymyxin Pyruvate Egg yolk Mannitol Bromothymolblue Agar) (base)
acc. ISO 21871

Ordering number: 1.20589.0500

For the selective isolation of viable presumptive *Bacillus cereus* from products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.

This culture medium complies with the specifications given by EN ISO 21871.

Mode of Action

This selective culture medium contains two indicator systems, mannitol and bromothymol blue and egg yolk. *B. cereus* does not utilize mannitol and forms precipitates of hydrolyzed lecithin from the egg yolk.

B. cereus grows with turquoise to peacock blue colonies, since the medium is slightly alkalized by its metabolites (bromothymol blue reaction). The yolk reaction (by lecithinase) also leads to a precipitate surrounding the colony, which appears in the color of the agar or similar to the colony. Growth and lecithinase activity are often so strong that the precipitation is already evident before a colony is visible.

B. anthracis, *B. mycoides*, *B. pseudodomycoides*, . B. *thuringiensis*, *B. weihenstephanensis* and *B. cytotoxicus* may not be distinguishable from *Bacillus cereus* on this medium.

Bacillus cereus is identified by colony form, color and egg yolk hydrolysis and rapidly confirmed by microscopic examination by its cell and spore morphology and the formation of intracellular lipid globules in vegetative cells. This staining method was developed by Holbrook and Anderson (1980) combining staining of spores and intracellular fatty material.

Mannitol-positive bacteria form yellow colonies with a yellow zone in the surrounding medium.

Due to a low concentration of nutrients and the selective inhibitory component polymyxin, selectivity is attained, but some *Proteus spp*. and gram-positive cocci can grow. Due to the low peptone level and the addition of pyruvate the egg yolk precipitation and the sporulation is improved. Bromothymol blue acts as a pH indicator to detect mannitol utilization. Enzymatic digest of casein is providing carbon and nitrogen sources, sodium chloride maintains the osmotic balance of the medium whilst agar is the solidifying agent.

Typical Composition

Specified by EN ISO 21871		**GranuCult® PEMBA (Polymyxin Pyruvate Egg yolk Mannitol Bromothymolblue Agar) (base) acc. ISO 21871**	
Enzymatic digest of casein	1 g/l	Enzymatic digest of casein	1 g/l
D-Mannitol	10 g/l	D(-)-Mannitol	10 g/l
Sodium pyruvate	10 g/l	Sodium pyruvate	10 g/l
Magnesium sulfate · 7 H_2O	0,1 g/l	Magnesium sulfate anhydrous*	0,049 g/l
Sodium chloride	2 g/l	Sodium chloride	2 g/l
Disodium hydrogen phosphate	2,5 g/l	Disodium hydrogen phosphate	2,5 g/l
Potassium dihydrogen phosphate	0,25 g/l	Potassium dihydrogen phosphate	0,25 g/l
Bromothymol blue	0,12 g/l	Bromothymol blue	0,12 g/l
Agar	9-18 g/l	Agar-Agar**	12,5 g/l
Water	940 ml/l	Water	n/a
Supplements after autoclaving:			
Polymyxin B sulfate	10^5 IU	Polymyxin B sulfate	10^5 IU
20% Egg yolk emulsion	50 ml	50% Egg yolk emulsion***	25 ml
pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.2 ± 0.2

* 0,049 g/l Magnesium sulfate anhydrous is equivalent to 0,1 g/l Magnesium sulfate · 7 H2O.

** Agar-Agar is equivalent to other different terms of agar.

*** EN ISO 21871 states that for usage of a commercial egg yolk emulsion, the concentration should be used according to the manufacturer's instruction

Preparation

Dissolve 19.21 g in 487.5 ml of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C).

At about 47-50°C mix in 12.5 ml of a sterile Egg-yolk emulsion (article number 103784) and the content of 1 vial Bacillus cereus Selective Supplement (article number 109875). Pour to plates.

The prepared medium is opalescent and yellowish-green.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure for detection and enumeration (by MPN) given by EN ISO 21871, inoculate PEMBA agar plates after selective enrichment in Tryptone soya polymyxin broth (TSPB).

Tryptone soya polymyxin broth (TSPB) can be prepared from Tryptic Soy Broth (article number 105459) with added Bacillus cereus Selective Supplement (article number 109875) after autoclaving.

From enrichment culture in TSPB, mix well and inoculate an inoculation loop streak the culture onto the surface of the PEMBA plates. Incubate the inoculated plates with the lid downwards at 37 °C ± 1 °C for 18 h to 24 h. If the colonies cannot be clearly assessed, continue incubating the plates for up to additional 24 h. For PEMBA this further incubation may also be carried out at room temperature.

After incubation is complete, examine the plates for the presence of typical or atypical colonies.

Presumptive colonies of *Bacillus cereus* on PEMBA are about 2 mm to 5mm in size, have an irregular edge which is in between ragged and root-like with ground glass surface, are turquoise to peacock blue (indicating that mannitol fermentation has not occurred), possibly with a greyish white colony centre against a blue background, and have a precipitation halo (egg yolk reaction) up to 5 mm (indicating the production of lecithinase).

Atypical colonies: If the plates have a high content of background flora which ferments mannitol, the characteristic coloration of the colonies and background may be reduced or no longer visible. In addition, some presumptive *Bacillus cereus* strains have only a slight egg yolk reaction or none at all. In such cases and in any other doubtful cases, these colonies should also be submitted to the confirmation.

Following EN ISO 21871, typical and atypical colonies on PEMBA shall be confirmed by means of the haemolysis test on sheep blood agar. Sheep blood agar can be prepared from GranuCult® Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM (article number 1.05458) with added sheep blood without fibrin after autoclaving.

Following confirmation by microscopic examination given by EN ISO 21871, typical and atypical colonies on PEMBA may be confirmed by means of microscopic examination using malachite green solution for staining the spores and Sudan black B solution for staining the intracellular fat globules and re-stain with safranin solution.

Examine the slide under a microscope using immersion oil. As a rule, the brick-shaped cells of presumptive *Bacillus cereus* are arranged in chains and are 4 µm to 5 µm long, 1 µm to 1,5 µm wide and contain fairly large amounts of intracellular fat which is stained black.

The green stained spores may be central or subterminal, but they never distend the red stained sporangia.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According EN ISO 21871, self-prepared plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to 4 days.

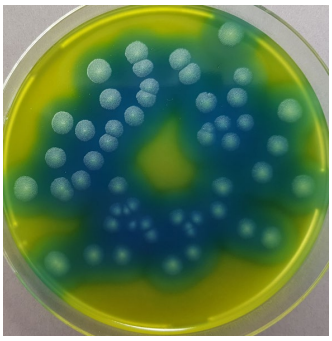
Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Bacillus cereus* ATCC® 11778 [WDCM 00001] *Bacillus cereus* ATCC® 13061 *Bacillus cytotoxicus* DSM 22905 [WDCM 00220]	18-24 h at 36-38 °C, aerobic	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥50 %, turquoise-blue colonies with precipitation halo
Selectivity	*Escherichia coli* ATCC® 8739 [WDCM 00012] *Escherichia coli* ATCC® 25922 [WDCM 00013]	40-44 h at 36-38 °C, aerobic	-	Qualitative	Total inhibition
Specificity	*Bacillus subtilis* subsp. *spizizenii* ATCC® 6633 [WDCM 00003]	40-44 h at 36-38 °C, aerobic	-	Qualitative	If growth: white colonies without precipitation halo

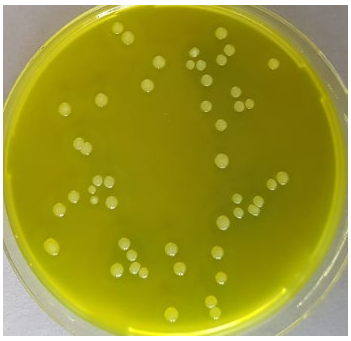
Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133 and EN ISO 21871.

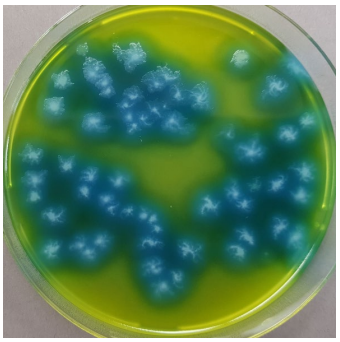
A recovery rate of 70 % is equivalent to a productivity value of 0.7.



Bacillus cereus ATCC 11778 (WDCM 00001)



Bacillus subtilis ATCC 6633 (WDCM 00003)



Bacillus cytotoxicus DSM 22905 (WDCM 00220)

Literature

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014+Amd1:2018.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the determination of low numbers of presumptive *Bacillus cereus* - Most probable number technique and detection method. EN ISO 21871:2006.

Becker, H., Schaller, G., Von Wiese, W. and Terplan, G. (1994) *Bacillus cereus* in infant foods and dried milk products. Int. J. Food Microbiol. **23**, 1–15.

Contzen, M., Hailer, M. and Rau J. (2014) Isolation of *Bacillus cytotoxicus* from various commercial potato products. Int. J. Food Microbiol. **174**, 19-22.

Fritze, D. and Pukall, R. (2012): Culture media for Bacillus spp. And Related Genera Relevant to Foods. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 90 – 114. Royal Society of Chemistry, Cambridge, UK.

Heini, N., Stephan, R., Ehling-Schulz, M. and Johler S. (2018) Characterization of *Bacillus cereus* group isolates from powdered food products. Int. J. Food Microbiol. **283**, 59-64.

Holbrook, R. and Anderson, J. M. (1980): An improved selective and diagnostic medium for the isolation of *Bacillus cereus* in foods. Can. J. Microbiol. **26**, 753-759.

Szabo, R.A., Todd, E.C.D. and Rayman, M.K. (1984): Twenty-Four Hour Isolation and Confirmation of *Bacillus cereus* in Foods. J. Food Prot. **47** (11), 856-860.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult® PEMBA (Polymyxin Pyruvate Egg yolk Mannitol Bromothymolblue Agar) (base) acc. ISO 21871	1.20589.0500	500 g	
Egg-Yolk Emulsion sterile, 50%, for microbiology	1.03784.0001	10 x 100 ml	
Bacillus Cereus Selective Supplement	1.09875.0010	10 x 1 vial	
ReadyPlate PEMBA ISO 21871	1.46711.0100	100 plates	
GranuCult™ MYP (Mannitol egg yolk polymyxin) agar (base) acc. ISO 7932, ISO 21871 and FDA-BAM	1.05267.0500	500 g	
ReadyPlate MYP ISO 7932, 21871, FDA-BAM	1.46160.0020	20 plates	100 plates
Tryptic Soy Broth (Casein-peptone soymeal-peptone broth for microbiology acc. harm. EP/USP/JP and ISO)	1.05459.0500	500 g	5 kg, 25 kg
GranuCult® Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM	1.05458.0500	500 g	5 kg
Certistain® Malachite green oxalate (C.I. 42000) for microscopy	1.15942.0025	25g	
Sudan Black B – certified by the Biological Stain Commission	199664-25G	25 g	
Certistain® Safranine O (C.I. 50240) for microscopy	1.15948.0025	25 g	

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