Technical Data Sheet

# GranuCult™
# MYP (Mannitol Egg Yolk Polymyxin) Agar (Base) acc. ISO 7932, ISO 21871 and FDA-BAM

Ordering number: 1.05267.0500

For the enumeration of presumptive *Bacillus cereus* from food and animal feed as well as from environmental samples and other materials.

This culture medium complies with the specifications given by EN ISO 7932, EN ISO 21871, FDA-BAM and APHA.

MYP (Mannitol egg yolk polymyxin) agar is also called Mannitol Egg Yolk Polymyxin (MEYP) agar.

## Mode of Action

This medium contains two indicator systems, mannitol and phenol red and egg yolk. Enzymatic digest of casein and meat extract are providing carbon and nitrogen sources. Due to the selective inhibitory component polymyxin, the growth of many unwanted microorganisms is suppressed.

Bacteria which ferment mannitol to produce acids produce a yellow staining of the medium with phenol red as a pH indicator. Bacillus cereus does not attack mannitol so the medium around the colonies remains unchanged or is discolored by light alkalization by forming pink colonies.

The lecithin present in egg yolk is cleaved by the *Bacillus cereus*-lecithinase, which leads to the formation of an opalescent white precipitation zone surrounding the pink colonies.

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

## Typical Composition

| Specified by EN ISO 7932, EN ISO 21871 | | Specified by BAM M95 | | GranuCult™ MYP (Mannitol egg yolk polymyxin) agar (base) acc. ISO 7932, ISO 21871 and FDA-BAM | |
|---|---|---|---|---|---|
| Beef extract | 1 g/l | Beef extract | 1 g/l | Meat extract* | 1 g/l |
| Enzymatic digest of casein | 10 g/l | Peptone | 10 g/l | Enzymatic digest of casein** | 10 g/l |
| D-Mannitol | 10 g/l | Mannitol | 10 g/l | D(-)Mannitol | 10 g/l |
| NaCl | 10 g/l | NaCl | 10 g/l | NaCl | 10 g/l |
| Phenol red | 0.025 g/l | Phenol red | 0.025 g/l | Phenol red | 0.025 g/l |
| Agar | 12-18 g/l | Agar | 15 g/l | Agar-agar*** | 12 g/l |
| Water | 900 ml/l | Water | 900 ml/l | Water | n/a |
| **Supplements after autoclaving:** | | | | | |
| Polymyxin B sulfate | $10^5$ IU | Polymyxin B sulfate | $10^5$ IU | Polymyxin B sulfate | $10^5$ IU |
| 20% Egg yolk emulsion | 100 ml/l | Egg yolk emulsion | 50 ml | Egg yolk emulsion | 100 ml/l |
| pH at 25 °C | 7.2 ± 0.2 | pH at 25 °C | 7.2 ± 0.2 | pH at 25 °C | 7.2 ± 0.2 |

* Meat extract is equivalent to the term beef extract.

** Enzymatic digest of casein is a peptone.

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

## Preparation

Dissolve 21.5 g in 450 ml of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave 15 minutes at 121 °C. At 47-50 °C mix in 50 ml (or 25 ml acc. FDA-BAM) of a sterile Egg-yolk emulsion (article number 103784) and the content of one vial *Bacillus cereus* Selective Supplement (article number 109875). Pour to plates.

The prepared medium is clear and red.

## Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

**Following the procedure for direct enumeration given by EN ISO 7932**, inoculate by means of a sterile pipette with 0.1 ml of the test sample or the initial dilution on each MYP agar plate. Repeat for further dilution if necessary.

Carefully spread the inoculum as quickly as possible over the surface of the agar plate, trying not to touch the sides of the dish, using a spreader. Allow the plates to dry with their lids on for about 15 min at the laboratory temperature.

Invert the inoculated plates and incubate them for 18-24 h at 29-31 °C.

**Following the procedure for enumeration and detection by MPN given by EN ISO 21871**, inoculate MYP agar plates by subculturing the 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.

**Presumptive colonies of *Bacillus cereus* on MYP agar** are large, pink (indicating that mannitol fermentation has not occurred) and generally surrounded by a zone of precipitation (indicating the production of lecithinase).

If the plates contain numerous mannitol-fermenting microorganisms leading to the production of acid, then the characteristic pink colour of presumptive *Bacillus cereus* colonies may be reduced or disappear entirely.

Some strains of *Bacillus cereus* produce only little or no lecithinase. Colonies of these strains will not be surrounded by a precipitation zone. These colonies also be subjected to confirmation tests.

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

For enumeration and confirmation follow the procedure e.g. given by EN ISO 7932 and EN ISO 21871.

## 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 to EN ISO 7932 and EN ISO 21871, self-prepared plates can be stored at +1 °C to +5 °C in the dark and protected against evaporation for up to four days.

## Quality Control

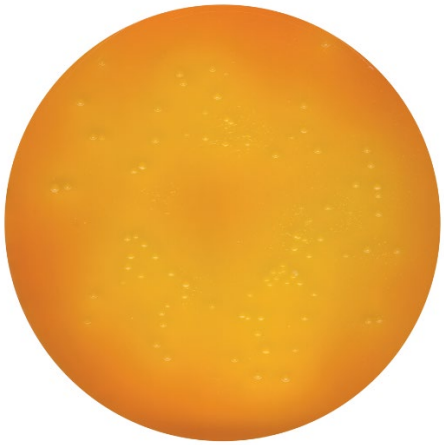
| Function | Control strains | Incubation | Reference medium | Method of control | Expected results |
|---|---|---|---|---|---|
| Productivity | *Bacillus cereus* ATCC® 11778 | 21-27 h to 40-48 h at 29-31 °C | Tryptic Soy Agar (TSA) | Quantitative | Recovery ≥ 50 %, Pink colonies with precipitation halo |
| Selectivity | *Escherichia coli* ATCC® 8739<br>*Escherichia coli* ATCC® 25922 | 40-48 h at 29-31 °C | - | Qualitative | Total inhibition |
| Specificity | *Bacillus subtilis* ATCC® 6633 | 40-48 h at 29-31 °C | - | Qualitative | Yellow 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. A

recovery rate of 50 % is equivalent to a productivity value of 0.5.

*Bacillus cereus* ATCC® 11778



*Staphylococcus aureus* ATCC® 6538

## Literature


APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

Donovan, K.O. (1958): A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. **21**: 100-103.

FDA-BAM (2013): Chapter No. 14: *Bacillus cereus*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

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 - 110. Royal Society of Chemistry, Cambridge, UK.

Harmon, S.M., Kautter, D.A. and McClure, F.D. (1984): Comparison of selective plating media for enumeration of *Bacillus cereus* in foods. J. Food Prot. **47**: 65-67.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 °C. EN ISO 7932:2004.

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

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.

Mossel, D.A.A., Koopman, M.J. and Jongerius, E. (1967): Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. **15**: 650-653.

## Ordering Information

| Product | Cat. No. | Pack size |
|---|---|---|
| GranuCult™ MYP (Mannitol Egg Yolk polymyxin) Agar (Base) acc. ISO 7932, ISO 21871 and FDA-BAM | 1.05267.0500 | 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 |
| Tryptic Soy Broth (Casein-peptone soymeal-peptone broth for microbiology acc. harm. EP/ USP/JP and ISO) | 1.05459.0500 | 500 g |
| ReadyPlate™ MYP ISO 7932, 21871 | 1.46160.0020 | 20 x 90 mm |
| ReadyPlate™ PEMBA ISO 21871 | 1.46711.0020 | 20 x 90 mm |
| Duopath® Cereus Enterotoxins | 1.04146.0001 | 25 tests |

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