

Mossel (MYP)/Agar (Enumeration of *Bacillus cereus*)

356-9604

DEFINITION

Medium used for the detection and enumeration of *Bacillus cereus* by the technique of colony count at 30°C in the analysis of food products.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 7932 (July 2005):** Microbiology - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony count technique at 30°C.

PRINCIPLE

The principle of the medium relies on the inability of *Bacillus cereus* to ferment mannitol (pink colonies) and on the manifestation of lecithinase.

Due to the presence of Polymyxin B sulfate, this medium inhibits other bacteria.

PRESENTATION

Dehydrated

500 g

code 356-9604

STORAGE

- + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	10 g
Meat extract	1 g
Sodium chloride	10 g
Mannitol	10 g
Phenol red	25 mg
Polymyxin B sulfate	10 ⁵ UI
Agar	14 g
Distilled water	900 ml

Final pH (25°C) = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- **Sterile egg-yolk solution**

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer

- 150 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø= 90 mm)
- Sterile pipettes (1 ml, etc)
- Sterile spreaders
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use

Dissolve 45 g of powder in 0,9 liter of distilled water and mix until a homogenous suspension is obtained.

Heat gently, swirling frequently, then bring to the boil until completely dissolved.

Dispense 90 ml per bottle and sterilize in autoclave at 121°C for 15 minutes.

Add 10 ml of a sterile 20% egg-yolk emulsion to the melted medium cooled to 44°C - 47°C.

Homogenize and pour in Petri dishes.

Reconstitution ratio: 45 g/0.9 l.

500 g of powder makes 10 liters of medium.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

• Inoculation and incubation

Transfer 0.1 ml of the sample to be analyzed to the surface of a Petri dish with the medium previously poured and dried.

Spread and incubate at 30°C ± 1°C for 18 - 24 hours.

If the colonies are not clearly visible, incubate for a further 24 hours.

READING AND INTERPRETATION

Count the presumptive *Bacillus cereus* colonies on each dish.

These are pink and are often surrounded by a zone of precipitate indicating production of lecithinase.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10⁻¹ dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.

- Do not add the egg-yolk to a base medium at a temperature exceeding 47°C.
- If the dishes contain numerous microorganisms producing acid from mannitol, the pink color characteristic of *B. cereus* colonies may be attenuated or may disappear completely.
- Certain strains of *B. cereus* produce little or no lecithinase. Colonies resulting from these strains will not be surrounded by a zone of precipitate. These colonies should also be subjected to confirmation tests.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Result of culture after 24h and 48h at 37°C
<i>Bacillus cereus</i> ATCC 9634	Good growth Pink colonies
<i>Bacillus subtilis</i> ATCC 6633	Good growth Yellow colonies
<i>Bacillus circulans</i>	Good growth Yellow colonies
<i>Bacillus licheniformis</i>	Good growth Yellow colonies
<i>Escherichia coli</i> ATCC 25922	Inhibition

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Mossel (MYP) / *Bacillus cereus* / Food products / Detection / Enumeration / Mannitol / Lecithinase / Medium.

BIBLIOGRAPHY

- **MOSSEL D.A.A, KOOPMAN M.J. and JONGERIUS E. (1967):** Enumeration of *Bacillus cereus* in foods. Applied Microbiology 15 (3) : 650-653
- **PANTALEON J. et coll:** Hygiène des denrées animales et d'origine animale. Techniques de laboratoire.