

Polymyxin B Supplement

PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

DESCRIPTION

MYP AGAR (Mannitol Egg Yolk Polymyxin Agar) with Polymyxin B Supplement is a medium for the enumeration of *Bacillus cereus* in food samples.

BACKGROUND

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa*. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

APPLICATION IN MANNITOL EGG YOLK POLYMYXIN AGAR

Bacillus cereus has been recognised as a causative agent of food poisonings since the 1950s. Many early isolation techniques used blood agar, relying on haemolysis and colony morphology for the detection of suspect *Bacillus cereus*, and then a range of tests for confirmation. The main problem with these media was that they were not selective and were generally only useful in detecting high numbers of *Bacillus cereus*.

Bacillus cereus is a Gram-positive, rod shaped, facultatively aerobic sporeforming organism. The cells are large (3.5µm) and sometimes form short chains or long strings with central to terminal ellipsoidal spores that do not distend the cell. Unstained globules within the cells occur when they are grown on glucose containing media. e

Although the presence of *Bacillus cereus* has been

determined in foods related to outbreaks of illness since the early years of the 20th century, its role as a causative agent in food poisoning was not established until the 1950s.

Two distinct syndromes, emetic and diarrhoeal, may occur in *Bacillus cereus* associated foodborne illnesses, related to two different metabolites. The diarrhoeal type of illness is caused by a heat labile, high molecular weight protein, while the vomiting (emetic) type is caused by a heat stable, low molecular weight peptide.

A wide range of foods have been associated with the diarrhoeal syndrome, including meat based dishes, soups, vegetables, puddings and sauces. The emetic syndrome seems to be associated to a more limited range of foods with approximately 95% of all cases related to fried or cooked rice. Foods implicated in *Bacillus cereus* induced illnesses usually contain at least 105 cfu/g, although about 10 % of outbreaks have been associated with food containing less than this. In almost all cases the implicated food has been held for too long at unsatisfactory storage temperatures.

MYP Agar is a selective and differential medium developed by Mossel et al.. The diagnostic features of the medium rely upon the failure of *Bacillus cereus* to utilise mannitol and the ability of most strains to produce phospholipase C. The medium is made selective by the addition of Polymyxin B which will inhibit Gram-negative bacteria. MYP Agar has proved to be very effective for detecting *B. cereus* even for ratios as challenging as one cell of *Bacillus cereus* to 10⁶ cells of other organisms.

Content concentrations

Typical Formula*	mg/litre
MYP Agar (Mannitol Egg Yolk Polymyxin Agar)	
Meat extract	1
Peptone	10
Mannitol	10
Sodium chloride	10
Phenol Red	0.025
Agar	12
Final pH 7.2 ± 0.2 @ 25°C	
Polymyxin B Supplement	
Polymyxin B	100,000 IU
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for MYP Agar (Mannitol Egg Yolk Polymyxin Agar) and Polymyxin B Supplement

METHOD

Preparation

Suspend appropriate amount of MYP Agar in distilled water and bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 49°C and aseptically add 50ml Egg Yolk Emulsion and Polymyxin B Supplement, reconstituted as directed. Mix well and pour into sterile petri dishes.

Protocol

1. Dry the surface of the agar medium. Prepare the food sample by making appropriate dilutions in Peptone Water.
2. Spread 0.1 ml of these dilutions over the surface of the agar plate using a sterile glass spreader.
3. Incubate at 30° C for 18-40 hours.
4. Count the number of typical colonies and calculate the viable count.
5. Typical colonies of *Bacillus cereus* are rough and dry with a bright pink background surrounded by an egg yolk precipitate.

Quality control

Positive control:

Bacillus cereus ATCC® 11778: Good growth; bright pink colonies; zone of egg yolk precipitation

Negative control:

Escherichia coli ATCC® 25922: No growth

REFERENCES

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