

TM 1843-MANNITOL EGG YOLK POLYMYXIN AGAR (ISO 7932:2004, ISO 21871:2006)

INTENDED USE

For the enumeration of *B. cereus* in foodstuffs and other samples.

PRODUCT SUMMARY AND EXPLANATION

Mannitol Egg Yolk Polymyxin 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 utilize mannitol and the ability of its most strains to show lecithinase activity. This medium also differentiates *B. cereus* on the basis of its resistance to Polymyxin B. This culture medium complies with the specifications given by EN ISO 7932, EN ISO 21871, FDA-BAM and APHA,

COMPOSITION

Ingredients	Gms / Ltr
Agar	12.000
Peptone	10.000
Mannitol	10.000
Sodium chloride	10.000
Meat extract	1.000
Phenol red	0.025

PRINCIPLE

The medium contains Peptone and meat extract which supply nitrogen and carbon. Sodium chloride provides the essential electrolytes in the medium. Agar is a solidifying agent. The medium is made selective by the addition of Polymyxin B which will inhibit Gram-negative bacteria. Mannitol and phenol red acts as an indicator system along with egg yolk. 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.

INSTRUCTION FOR USE

- Dissolve 43.02 grams in 900ml distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes
- Cool to 45-55°C.
- Aseptically add 2 vials of Polymyxin B Selective Supplement (TS 058) and 100ml Egg Yolk Emulsion (TS 002) per 1000 ml of medium.
- Mix well and pour into petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder	:	Light yellow to light pink, homogeneous free flowing powder
Appearance of Prepared medium	:	
Basal medium	:	Red colored, clear to slightly opalescent gel
After addition of Egg yolk emulsion (TS 002)	:	Light orange colored, opaque gel
pH (at 25°C)	:	7.2± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with addition of Polymyxin B selective supplement (TS 058) and Egg yolk emulsion (TS 002. Recovery rate is considered 100% for bacteria growth on Soya Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Lecithinase activity	Incubation Temp.	Incubation Period
<i>Bacillus cereus</i>	10876	50-100	Luxuriant	>=50%	Red	Positive Reaction (opaque zone around colony)	30-32°C	18-48 Hours
<i>Bacillus subtilis</i>	6633	50-100	Luxuriant	>=50%	Yellow	Negative	30-32°C	18-48 Hours
<i>Staphylococcus aureus</i>	25923	50-100	Luxuriant	>=50%	Yellow	Positive Reaction (opaque zone around colony)	30-32°C	18-48 Hours
<i>Proteus mirabilis</i>	25933	50-100	Luxuriant	>=50%	Red	Negative	30-32°C	18-48 Hours
<i>Pseudomonas aeruginosa</i>	27853	50-100	None-Poor	<= 10%	-	-	30-32°C	18-48 Hours
<i>Escherichia coli</i>	25922	50-100	None-Poor	<= 10%	-	-	30-32°C	18-48 Hours

PACKAGING:

In 500 gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. ISO International Standardization organization. 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
2. Mossel, D.A.A., Koopman, M.J. and Jongerius, E. (1967): Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15: 650-653.
3. ISO International Standardization Organization. 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
4. Jenson, I. and C. J. Moir (1997) *Bacillus cereus* and other *Bacillus* species. In: Foodborne Microorganisms of Public Health Significance. 5th Edition. pp.379-406. A. D. Hocking (Ed.). AIFST (NSW Branch) Food Microbiology Group, Australia.

