



Soyabean Casein Digest Medium Base w/o Polymyxin

M011F

Intended use

Soyabean Casein Digest Medium Base with added Polymyxin is recommended for selective isolation and MPN method of *B.cereus*. The composition and performance criteria are as per the specification laid down in FDA BAM, 1998, ISO 21871 and ISO 11133:2014 & Amd.2:2020 (E).

Composition**

ISO 21871 : Tryptone Soya Polymyxin Broth

Ingredients	g / L
Tryptone #	17.000
Soya peptone ##	3.000
Sodium chloride	5.000
Dextrose	2.500
Dibasic potassium phosphate	2.500
Final pH (at 25°C)	7.3±0.2

M011F: Soyabean Casein Digest Medium Base w/o Polymyxin

Ingredients	g / L
Tryptone #	17.000
Soya peptone ##	3.000
Sodium chloride	5.000
Dextrose	2.500
Dipotassium hydrogen phosphate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein

Papaic digest of soyabean meal

Directions

Suspend 30 gram in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Add one vial of sterile PolyB Sulphate (FD003) solution to a final concentration of 100 Units/ml. Mix well and dispense as desired.

Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.

Principle And Interpretation

Bacillus cereus is a large, 1 × 3-4 µm, Gram-positive, rod-shaped, endospore forming, and facultative aerobic bacterium. They are mesophilic and can grow in a wide range of environments and are commonly found in nature, vegetables and in several processed foods. Under favorable circumstances the microorganism grows to sufficient numbers and cause gastrointestinal illness. Outbreaks of food borne illness have been associated with boiled and cooked rice, cooked meat and vegetables (1). The infection mediates diarrhoeal illness that is attributed by a heat and acid labile enterotoxin. Soyabean Casein Digest Medium Base (SCDM) with polyB (FD003) is recommended for the selective isolation and MPN method of *Bacillus cereus* in accordance with FDA BAM(2) and ISO (3,4) for selective enrichment of *B.cereus*. *B.cereus* in general is resistant to polyB and the addition of it into the medium helps in the selective isolation of the organism.

Without supplement, SCDM is a highly nutritious medium used for cultivation of a wide variety of organisms (3). The combination of tryptone and soya peptone makes the medium nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Dextrose and dipotassium hydrogen phosphate serve as the carbohydrate source and the buffer, respectively in the medium. Sodium chloride maintains the osmotic balance of the medium. FDA BAM suggests two methods to check the presence of *B.cereus* that are 1) Serial dilution method and 2) MPN Method.

According to the serial dilution protocol, appropriate dilutions of the suspected samples are made in Butterfield's Phosphate Buffered Dilution Water (R094) and spread plate was done with 0.1 ml of respective dilutions in MYP Agar Base (M636F). According to the MPN method, 1 ml each of 10⁻¹, 10⁻² and 10⁻³ are inoculated into Soyabean Casein Digest Medium Base (M011F) with polyB (FD003) incubate for 48 ± 2 h at 30 ± 2°C. Observation of turbid growth after the incubation time is indicative of the presence of *B.cereus*. Positive cultures are further inoculated into MYP Agar Base (M636F) and incubated 18-24 h at 30°C. *B.cereus* appears as pink coloured colonies surrounded by a precipitate zone of lecithinase activity. Biochemical tests are performed to confirm the species.

According to ISO, selective enrichment is carried out in M011F and isolated on PEMBA Agar (M1484).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (2-5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate.

Reaction

pH of 3.0% w/v aqueous solution at 25°C . pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Productivity : Cultural characteristics observed by adding PolyB Selective Supplement (FD003), after an incubation at 30 ±1°C for 48± 4 hours.

Selectivity : Cultural characteristics observed by adding PolyB Selective Supplement (FD003), after an incubation at 30 ±1°C for 48± 4 hours.

Organism	Inoculum (CFU)	Growth
Productivity		
<i>Bacillus cereus</i> ATCC 10876	50-100	luxuriant
Selectivity		
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ⁴	inhibited

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1.Hoffmaster, A., Hill, K., Gee, J., Marston, C., De, B., Popovic, T., Sue, D., Wilkins, P., Avashia, S., Drumgoole, R., Helma, C., Ticknor, L., Okinaka, R. and Jackson, J 2006. Journal of clinical microbiology, 44(9): 3352-3360.

2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Microbiology of food, animal feeding stuffs and water- Preparation, production, storage and performance testing of culture media, EN ISO 11133:2014 /Amd.2 : 2020 (E) .
4. 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, ISO 21871:2006
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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