



Technical Data

Bacillus Differentiation Agar

M1394

Intended Use:

Recommended for differentiation between *Bacillus cereus* and *Bacillus subtilis* based on mannitol fermentation.

Composition**

Ingredients	Gms / Litre
Yeast autolysate	0.200
Mannitol	5.000
Monohydrogen ammonium phosphate	1.000
Potassium chloride	0.200
Magnesium sulphate	0.200
Bromo cresol purple	0.0075
Agar	15.400
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Bacillus is Gram positive, rod-shaped bacteria; can be obligate aerobes or facultative anaerobes (4). Under stressful environmental conditions they produce oval endospores, that can be dormant for extended periods (3). *Bacillus cereus* causes food-borne illness and *Bacillus subtilis* is involved in food spoilage like ropiness in bread and other related foods. Bacillus Differentiation Agar is recommended for differentiation between *Bacillus cereus* and *Bacillus subtilis* based on mannitol fermentation. Yeast autolysate provide necessary nitrogenous source for growth of *Bacillus*. Magnesium sulphate and Potassium chloride supports sporulation. Ammonium phosphate maintains buffering action. Bromocresol purple act as a pH indicator to detect mannitol fermentation.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

For samples follow appropriate techniques for handling specimens as per established guidelines (1,2). 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. Well isolated colonies must be used.

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

Light yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.54 % Agar gel.

Colour and Clarity of prepared medium

Light purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.2% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour
<i>Bacillus cereus</i> ATCC 10876	50-100	luxuriant	≥70%	colourless
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633 (00003*)	50-100	luxuriant	≥70%	yellow

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. 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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Madigan M; Martinko J (editors). (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall.
4. Turnbull PCB (1996). *Bacillus*. In: Barron's Medical Microbiology (Baron S et al., eds.) (4th ed.). Univ of Texas Medical Branch.

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Disclaimer :

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