



Bacillus Differentiation Agar

M1394

This medium is used for the 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 distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Bacillus is Gram positive, rod-shaped bacteria; can be obligate aerobes or facultative anaerobes(1). Under stressful environmental conditions they produce oval endospores, that can be dormant for extended periods (2). *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.

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

M1394: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour
Cultural Response				
<i>Bacillus cereus</i> ATCC 10876	50-100	luxuriant	≥70%	colourless
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	≥70%	yellow

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8 °C. Use before expiry date on label.

Reference

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

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