

TECHNICAL DATA SHEET

Bacillus Differentiation Agar

Principle

Bacillus are Gram positive, rod-shaped, obligate aerobes or facultative anaerobic bacteria. The most of **bacillus** are responsible for food spoilage and the food borne illness. As these **bacillus** species are endospore forming have ability to be dormant for the extended periods. The *Bacillus cereus* is one of the foods born illness causing organism and *Bacillus subtilis* is responsible for the food spoilage like bread and bakery products. The **bacillus** differentiation agar differentiates the based on the mannitol fermentative capability of the *Bacillus cereus* and *Bacillus subtilis*. The *Bacillus subtilis* ferments mannitol and reduces the pH of the medium resulting in the yellow-colored colonies; while the *Bacillus cereus* does not ferment mannitol resulting in the colorless colonies.

Use: For the differentiation between *Bacillus cereus* and *Bacillus subtilis* based on mannitol fermentation.

Contents*

Ingredients	Gram/Litre
Yeast autolysate	0.200
Mannitol	5.000
Monohydrogen ammonium phosphate	1.000
Potassium chloride	0.200
Magnesium sulphite	0.200
Bromocresol Purple	0.0075
Agar	15.400
pH at 25°C	7.2 ±0.2

*** Formula adjusted for optimum performance and parameters**

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Directions: Dissolve 22.00 grams in 1000 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 min, cool it to 42-45 °C, mix well and pour into sterile petri plates. Ensure complete solidification and inoculate test sample aseptically.

Specimens types analyzed

Food and food product, water, clinical and non-clinical isolates

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Greenish beige colored free flowing, homogeneous powder
Reaction of 2.2% solution	7.2 ±0.2 at 25 °C
pH	7.00- 7.40
Gelling	Firm comparable with 1.54% agar gel
Color and clarity of ready medium	Light purple colored clear to slightly opalescent gel.
Growth Promotion properties	Best at ≤ 100 CFU at 32-37°C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

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Different Microbial Response: Prepare media as per the label directions. Inoculate and incubate the plates at $35 \pm 2.0^{\circ}\text{C}$ for 18-24 hours. Inoculum 50-100 CFU.

Organism	ATCC	Growth	Recovery	Color of colony
<i>Bacillus cereus</i>	10876	Luxuriant	$\geq 70\%$	Colorless
<i>Bacillus subtilis</i>	6633	Luxuriant	$\geq 70\%$	Yellow

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Brown, J. H. (1919). The use of blood agar for the study of streptococci, NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
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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