



Technical Data

HiCrome Bacillus Agar

M1651

HiCrome Bacillus Agar is recommended for isolation and differentiation between various species of *Bacillus* from a mixed culture by chromogenic method.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Meat extract	1.000
D-Mannitol	10.000
Sodium chloride	10.000
Chromogenic mixture	3.200
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 49.22 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. Cool to 45-50°C. For selective isolation of *B.cereus* and *B.thuringiensis* aseptically add rehydrated contents of 1 vial of Bacillus Selective Supplement (FD324). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Majority of *Bacillus* species apparently have little or no pathogenic potential and are rarely associated with disease in humans or lower animals. The principal exception to this are *Bacillus anthracis*, the agent of anthrax, and *Bacillus cereus*, but a number of other species, particularly those of the *B.subtilis* group, have been implicated in food poisoning and other human and animal infections (1). *Bacillus cereus* causes food poisoning due to consumption of contaminated rice (3, 4, 5), other starchy foods such as potato, pasta and cheese have also been implicated, eye infections and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection.

HiCrome Bacillus Agar is based on the formulation of MYP Agar formulated by Mossel et al (2) used for enumeration of *Bacillus cereus* and *Bacillus thuringiensis* when present in large number in certain foodstuffs.

The medium contains peptic digest of animal tissues and meat extract, which provide nitrogenous compounds. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. Mannitol fermenting organisms like *B. megaterium* yield yellow coloured colonies. The chromogenic mixture present in the medium is cleaved by the enzyme beta-glucosidase found in *B.cereus* resulting in the formation of blue colonies. *B.thuringiensis* also grows as blue/green colonies on this medium as *B.cereus* and *B.thuringiensis* are biochemically identical, however *B.cereus* shows flat colonies with distinct blue centres, while *B.thuringiensis* shows irregular margins. If selective isolation of *B.cereus* or *B.thuringiensis* is required aseptically add selective supplement (FD324).

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.92% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Please refer disclaimer Overleaf.

Cultural Response

Cultural characteristics observed after an incubation at 30°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth w/o addition of FD324	Recovery w/o addition of FD324	Growth w/ addition of FD324	Recovery w/ addition of FD324	Colour of Colony
Cultural Response						
<i>Bacillus subtilis</i> ATCC 6633	50-100	fair	20-30%	inhibited	0%	yellowish green to green colonies
<i>Bacillus cereus</i> ATCC 10876	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue, large, flat colonies with blue centre
<i>Bacillus thuringiensis</i> ATCC 10792	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue, large, flat colonies with irregular margins
<i>Bacillus megaterium</i> ATCC 14581	50-100	good-luxuriant	>=50%	inhibited	0%	yellow, mucoid colonies
<i>Bacillus coagulans</i> ATCC 7050	50-100	good-luxuriant	>=50%	inhibited	0%	pink, small, raised colonies
<i>Bacillus pumilis</i> ATCC 14884	50-100	good-luxuriant	>=50%	poor	10-20%	light green to green colonies
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant	>=50%	inhibited	0%	yellow colonies
<i>Enterococcus faecalis</i> ATCC 29212	50-100	luxuriant	>=50%	inhibited	0%	light green to green colonies

Storage and Shelf Life

Store dehydrated powder in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed. American Society for Microbiology, Washington, D.C.
2. Mossel D. A. A., Koopman M. J. and Jongerius E., 1967, Appl. Microbiol., 15:650.
3. Mortimer P. R. and McCann G., 1974, Lancet, 1043.
4. Bouza E., Grant S., Jordan C. et al, 1979, Arch. Ophthalmol., 97:488.
5. Wohlgenuth K., Kirkbride C. A., Bicknell E. J. and Ellis R. P., 1972 Am. Vet. Med. Ass. 161:1691.

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