HiCrome™ Bacillus Agar

M1651

Intended use

Recommended for isolation and differentiation between various species of *Bacillus* from a mixed culture in foods, clinical and non-clinical samples by chromogenic method.

Composition**

Ingredients	g / L
Peptone	10.000
HM 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
#-Equivalent to Meat extract

Directions

Suspend 49.22 grams in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Cool to 45-50°C For selective isolation of *B.cereus* and *B.thuringiensis*, aseptically add rehydrated contents of one vial of PB 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 (2,3,4), 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 (5) used for enumeration of *Bacillus cereus* and *Bacillus thuringiensis* when present in large number in certain foodstuffs.

The medium contains peptone and HM extract, which provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. 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 centers, while *B.thuringiensis* shows irregular margins. If selective isolation of *B.cereus* or *B.thuringiensis* is required aseptically add PB Selective Supplement (FD324).

Type of specimen

Clinical samples - wounds, faeces, etc.; Food and dairy samples; Soil samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6-8).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

For Soil samples follow appropriate techniques for handling specimens as per established guidelines (11).

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

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical 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.
3. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

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 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

Cultural Response

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

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
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	fair	20-30%	inhibited	0%	yellowish green to green colonies
Bacillus cereus ATCC 10876	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue,large,flat colonies with blue centre
Bacillus thuringiensis ATCC 10792	50-100	good-luxuriant	>=50%	good-luxuriant	>=50%	light blue,large,flat colonies with irregular margins
$ Priestia megaterium ATCC 14581	50-100	good-luxuriant	>=50%	inhibited	0%	yellow, mucoid colonies
^*Weizmannia coagulans* ATCC 7050 (00002*)	50-100	good-luxuriant	>=50%	inhibited	0%	pink,small, raised colonies
Bacillus pumilis ATCC 14884	50-100	good-luxuriant	>=50%	poor	10-20%	light green to green colonies
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=50%	inhibited	0%	yellow colonies
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	inhibited	0%	light green to green colonies

Key: (*) - Corresponding WDCM numbers, **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

($)-Formerly known as *Bacillus megaterium.* (^)-Formerly known as *Bacillus coagulans*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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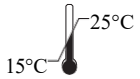
HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged