**HiCrome™ Bacillus Agar Plate**

**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**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HM extract #</td>
<td>1.000</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.000</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>3.200</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Bacillus Selective supplement (FD324)**

- Polymyxin sulphate                  | 10.000 mg   |
- Bacitracin                           | 10.000 mg   |

**Final pH (at 25°C)**: 7.1±0.2

**Directions**

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**Principle And Interpretation**

Majority of *Bacillus* species apparently have little or no pathogenic potential and are rarely associated with disease in human or lower animals. The principal exception to this are *Bacillus anthracis*, the agent of anthrax, and *Bacillus cereus*, but a small number of other species, particularly those of the *B.subtilis* group, have been implicated in food poisoning and other human and animal infections (7). *Bacillus cereus* causes food poisoning due to consumption of contaminated rice (2,5,10), 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 (6) 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. 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. Bacillus Selective supplement (FD324). inhibits the growth of other *Bacillus* and contaminating microflora.

**Type of specimen**

Food and dairy samples.

**Specimen Collection and Handling:**

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,9).

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

**Warning and Precautions:**

Read the label before opening the pack. 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. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. 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.

### 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
Sterile HiCrome™ Bacillus Agar in 90 mm disposable plates.

#### Colour of medium
Red coloured medium

#### Quantity of medium
25 ml of medium in 90 mm disposable plates.

#### pH
6.90-7.30

#### Sterility Test
Passes release criteria

### Cultural Response

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis subsp.</em></td>
<td>50-100</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>spizizenii ATCC 6633</em></td>
<td>(0003*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus ATCC 10876</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>light blue, large, flat colonies with blue centre</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis ATCC</em></td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>light blue, large, flat colonies with irregular margins</td>
</tr>
<tr>
<td><em>10792</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus megaterium ATCC</em></td>
<td>50-100</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>14581</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus coagulans ATCC</em></td>
<td>50-100</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>7050 (0002</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus pumilis ATCC</em></td>
<td>50-100</td>
<td>poor</td>
<td>10-20%</td>
<td>light green to green colonies</td>
</tr>
<tr>
<td><em>14884</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Staphylococcus aureus subsp.</em></td>
<td>50-100</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>aureus ATCC 25923 (00034</em>)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis ATCC</em></td>
<td>50-100</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>29212 (00087</em>)</td>
<td></td>
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</tr>
</tbody>
</table>

* - Corresponding WDCM numbers

### Storage and Shelf Life

On receipt store between 2-8°C Use before expiry date on the label. Product performance is best if used within stated expiry period.

Please refer disclaimer Overleaf.
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 (3,4).

Reference

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