HiCrome™ Coliform Agar Plate, Modified

**Intended Use:**
Recommended as a selective medium for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>8.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.600</td>
</tr>
<tr>
<td>Bile Salts</td>
<td>0.200</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.800</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>0.200</td>
</tr>
<tr>
<td>Agar</td>
<td>10.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Directions**

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

**Principle And Interpretation**

HiCrome™ Coliform Agar Modified is a selective medium recommended for the simultaneous detection of *E.coli* and thermo-tolerant coliforms in water and food samples (8). Peptone special and yeast extract provide carbon, nitrogen compounds, long chain amino acids, vitamins and essential growth nutrients to the organisms. The phosphates buffer the medium well. Magnesium sulphate helps colour development. Bile salts inhibits gram-positive organisms. Sodium chloride maintains osmotic balance. The chromogenic mixture contains two chromogenic substrates, which enables the detection of two specific enzymes, β-galactosidase and β-glucuronidase. β-galactosidase produced by coliforms cleaves one chromogen, resulting in the pink colouration of coliform colonies. The enzyme β-glucuronidase produced by *E.coli*, cleaves X-glucuronide. *E.coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (3,6,7). *E.coli* strains that are β-glucuronidase negative (serotype O157:H7) produce pink coloured colonies. Other gram negative bacteria able to grow at (44±0.5 ºC) produce white or colourless colonies. Transfer 1 ml of product to analyze and its tenfold dilutions to sterile Petri plates. Pour 12 ml of medium, mix well and allow to solidify. Overlay with 4 ml of medium, allow to solidify and incubate at 43-45°C for 18-24 hours.

**Type of specimen**

Food and Dairy samples; Water samples.

**Specimen Collection and Handling**

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1.9.10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2).

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

**Warning and Precautions**

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 specimens. Safety guidelines may be referred in individual safety data sheets.

---

Please refer disclaimer Overleaf.
Limitations
1. β-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. 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™ Coliform Agar Modified in 90 mm disposable plates.

Colour of medium
Light yellow to yellow coloured medium

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

pH
7.00-7.40

Sterility Test
Passes release criteria

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24 hours (48 hours if necessary).

<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>Salmonella Enteritidis</em> ATCC 13076 (00030*)</td>
<td>50-100</td>
<td>good</td>
<td>40-50%</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em> ATCC 23355</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pink</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>light pink</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 10536</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>dark blue/violet</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>dark blue/violet</td>
</tr>
</tbody>
</table>

Key : *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.

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 (4,5).

Reference

Please refer disclaimer Overleaf.

Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.