Rapid HiColiform™ HiCynth™ Broth

**Intended use**
Recommended for detection and confirmation of *Escherichia coli* and total coliforms from water samples, using a combination of chromogenic and fluorogenic substrates.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiCynth™ Peptone No.1#</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.700</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium lauryl sulphate (SLS)</td>
<td>0.100</td>
</tr>
<tr>
<td>Chromogenic substrate</td>
<td>0.080</td>
</tr>
<tr>
<td>Fluorogenic substrate</td>
<td>0.050</td>
</tr>
<tr>
<td>Isopropyl-β-D-1-thiogalactopyranoside (IPTG)</td>
<td>0.100</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 16.03 grams in 1000 ml purified / distilled water. For double strength broth use 32.06 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and dispense as desired.

**Principle And Interpretation**
Rapid HiColiform™ HiCynth™ Broth is a modification of LMX Broth described by Manafi and Kneifel (2). This medium is useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (2-9). It replaces animal and vegetable peptones with synthetic peptones to avoid BSE/TSE, GMO risks.

The fluorogenic substrate is split by enzyme β-D-glucuronidase specifically found in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of β-D-galactosidase. To confirm presence of *E.coli* overlay the medium with Kovacs reagent. The layer turns red within 2 minutes in case of positive reaction.

HiCynth™ Peptone No.1 serves as a source of carbon and nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients. Sorbitol is the fermentable carbohydrate. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms.

**Type of specimen**
Food samples; Water samples.

**Specimen Collection and Handling**
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).
After use, contaminated materials must be sterilized by autoclaving before discarding.

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Please refer disclaimer Overleaf.
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.

Limitations:
1. 97% of the E.coli strains are β-D-glucuronidase positive, however few strains may show negative fluorescence.

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
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured, clear solution having slight precipitate in tubes

Reaction
Reaction of 1.60% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Colour of Medium</th>
<th>Fluorescence (under uv)</th>
<th>Indole reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td># Klebsiella aerogenes</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue-green</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>ATCC 13048 (00175*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue-green</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>25922 (00012*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# Formerly known as Enterobacter aerogenes

Key : *Corresponding WDCM numbers.

Storage and Shelf Life
Store dehydrated 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference