**HiCrome™ Enterobacter sakazakii Agar, Modified**  
(HiCrome™ Cronobacter sakazakii HiVeg™ Agar, Modified)  
(*Enterobacter sakazakii now referred as Cronobacter sakazakii*)

**Intended Use:**
Recommended for the isolation and identification of *Cronobacter sakazakii* from milk, milk products and clinical samples.

Key: *: Formerly known as *Enterobacter sakazakii*

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ hydrolysate</td>
<td>7.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Synthetic detergent No. III</td>
<td>0.600</td>
</tr>
<tr>
<td>Chromogenic substrate</td>
<td>0.150</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 30.75 grams in 1000 ml purified / 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 and pour into sterile Petri plates.

**Principle And Interpretation**
*Enterobacter* species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human faeces. *Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (1). HiCrome™ Enterobacter sakazakii Agar, Modified is recommended by ISO Committee for the isolation and identification of *C.sakazakii* (2). The chromogenic substrate is cleaved specifically (3) by *C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colourless to slightly violet coloured colonies. HiCrome™ Cronobacter sakazakii HiVeg™ Agar, Modified is same as HiCrome™ Cronobacter sakazakii Agar, Modified except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones. HiVeg™ hydrolysate and yeast extract provide the essential growth nutrients along with nitrogenous and carbonaceous compounds. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Synthetic detergent No. III and crystal violet inhibits the accompanying gram-positive flora.

Key: *: Formerly known as *Enterobacter sakazakii*

**Type of specimen**
Clinical samples - Blood, cerebrospinal fluid, Food and Dairy samples; Water and wastewater samples

**Specimen Collection and Handling**
For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4, 5, 8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6).

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

**Warning and Precautions**
In Vitro diagnostic use. 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.

Please refer disclaimer Overleaf.
Limitations
1. Some species may show poor growth due to nutritional variations.

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
Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.07% w/v aqueous solution at 25°C. pH: 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 44±1°C for 18-24 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>Enterobacter aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless with blue centre.</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212 (00087*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless</td>
</tr>
<tr>
<td>*Cronobacter sakazakii ATCC 12868</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>blue-green</td>
</tr>
</tbody>
</table>

Cultural Response

Key: *Corresponding WDCM numbers.

Storage and Shelf Life
Store dehydrated powder and prepared medium on receipt at 2-8°C. Use before expiry period 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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,7).

Reference
3. Isenberg, (2nd Ed.), Clinical Microbiology Procedures handbook, Volume 1, American Society for Microbiology, Washington, D.C.

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.