



## HiCrome™ Cronobacter Isolation Agar (CCI Agar)

M2062I

### Intended Use

Recommended for the isolation and identification of *Cronobacter sakazakii* from food products. The composition and performance of this media are as per specifications laid down in ISO 22964: 2017.

### Composition

#### ISO 22964: 2017 Specification - Chromogenic Cronobacter isolation (CCI) agar

Ingredients	g / L
Tryptic digest of casein	7.000
Yeast extract	3.000
Sodium chloride (NaCl)	5.000
Sodium desoxycholate (C <sub>24</sub> H <sub>39</sub> NaO <sub>4</sub> )	0.250
5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside	0.15
Ammonium iron(III) citrate (C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> FeNH <sub>3</sub> )	1.000
Sodium thiosulfate (N <sub>2</sub> S <sub>2</sub> O <sub>3</sub> )	1.000
Agar	9.00-18.00
Final pH after sterilization ( at 25°C)	7.3±0.2

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Yeast extract	3.000
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Ammonium iron(III) citrate	1.000
Sodium thiosulfate	1.000
Agar	15.000
Final pH after sterilization ( at 25°C)	7.3±0.2

\*\*Formula adjusted, standardized to suit performance parameters

# - Equivalent to Tryptic digest of casein

### Directions

Suspend 32.4 gram 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. Mix well 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 (1,2). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of \**C.sakazakii* from food samples (3). The chromogenic substrate (5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside) is cleaved specifically by \**C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colourless colonies. Tryptone and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Sodium desoxycholate inhibits the accompanying gram-positive flora.

Key: \*: Formerly known as *Enterobacter sakazakii*

### Type of specimen

**ISO 22964: 2017:** Food products and ingredients intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.

### Specimen Collection and Handling:

**Processing : ISO 22964: 2017**

**Non-selective pre-enrichment in BPW (M1494I) :** Samples (10 gram/ 10ml in 90 ml) are pre-enriched in Buffered Peptone Water and incubated at 34 °C and 38 °C for 18 ± 2 hours.

**Selective enrichment (CSB) :** 0.1 ml of enriched culture from BPW (M1494I) is then inoculated into Cronobacter Selective Broth (CSB) and incubated at 41,5 °C ± 1 °C for 24 ± 2 hours.

**Identification on chromogenic agar (CCI agar) :-** 10 microlitre of selectively enriched culture from CSB (M1786I) is then cultured onto CCI Agar (M2062I) and incubated at 41,5 °C ± 1 °C for 24 ± 2h.

**Confirmation :** Typical colonies are selected from the chromogenic agar, purified on a non-selective agar such as TSA and biochemically characterized. 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.

## Limitations

1. Slight variation in colour may be observed depending on enzyme production by organism and substrate utilization from the medium.
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.
4. Further biochemical tests must be carried out for confirmation.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored recommended temperature.

## Quality Control

### Appearance

Cream to yellow to pink homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 3.24% w/v aqueous solution at 25°C. pH : 7.3±0.2

### pH

7.10-7.50

### Cultural Response

Cultural characteristics observed after an incubation at 41.5±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Inoculum (CFU)	Colour of Colony
<b>Productivity</b>				
<i>Cronobacter sakazakii</i> ATCC 29544 (00214*)	50-100	good	≥50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
<i>Cronobacter muytjensii</i> ATCC 51329 (00213*)	50-100	good	≥50%	blue to blue-green colonies (small to medium sized, 1 -3mm)
<b>Selectivity</b>				
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 <sup>4</sup>	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	≥10 <sup>4</sup>	inhibited	0%	
<b>Specificity</b>				
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	10 <sup>3</sup> -10 <sup>4</sup>	growth or partial inhibition		Colonies without green or blue green colour

Key: (\*) Corresponding WDCM numbers

## 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,4).

## Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2<sup>nd</sup> Edition.
2. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983
3. Microbiology of the food chain- Horizontal method for the detection of *Cronobacter* spp. International Organization for Standardization.Draft ISO/ TS 22964, 2017 (E).
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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### Disclaimer :

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