

For the isolation and identification of *Cronobacter sakazakii*

HiCrome™ Cronobacter Isolation Agar (CCI Agar)

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 in ISO /TS 22964: 2017

M2062I

Composition **

Ingredients	Grams/Litre
Tryptone#	7.00
Yeast extract	3.00
Sodium chloride	5.00
Sodium deoxycholate	0.25
5-Bromo-4-chloro-3-indolyl α -D-glucopyranoside	1.50
Ammonium iron(III) citrate	1.00
Sodium thiosulfate	1.00
Agar	15.00

Final pH (at 25°C) 7.3 \pm 0.2

** Formula adjusted, standardized to suit performance parameters

- Equivalent to Tryptic digest of casein

Directions

Suspend 32.4 grams in 1000 ml 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). HiCrome™ Cronobacter isolation Agar is recommended by ISO Committee for the isolation and identification of **C.sakazakii* from food samples (2).

The chromogenic substrate (5-Bromo-4-chloro-3-indolyl α -D-glucopyranoside) 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 coloured 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 deoxycholate inhibits the accompanying gram-positive flora.

Key: #: Formerly known as *Enterobacter sakazakii*.

Type of specimen

Food samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2, 3).

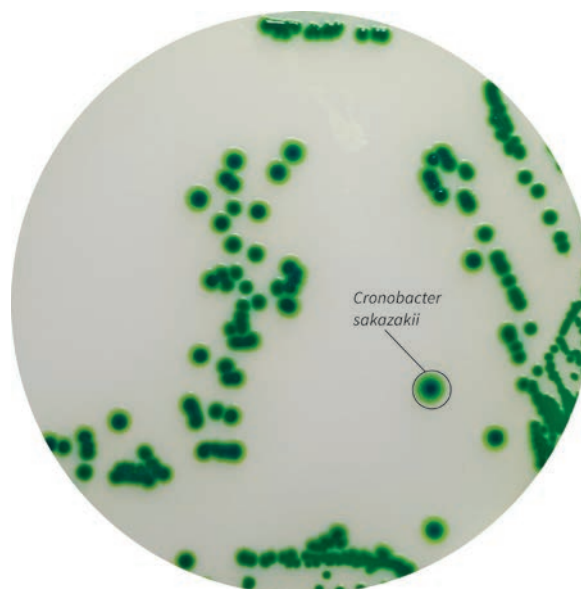
After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Some species may show poor growth due to nutritional variations.
3. Further biochemical tests must be carried out for confirmation.



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Performance and Evaluation

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

Quality Control

- Appearance of Powder** : Cream to yellow to pink homogeneous free flowing powder.
- Gelling** : Firm, comparable with 1.5% Agar gel
- Colour and Clarity** : 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
- Cultural Response** : Cultural characteristics observed after an incubation at 41.5±1°C for 24±2 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
* <i>Cronobacter sakazakii</i> (29544) (00214*)	50-100	good-luxuriant	>=50%	blue-green
<i>Cronobacter muytjensii</i> (51329) (00213*)	50-100	good-luxuriant	>=50%	blue-green
<i>Enterobacter cloacae</i> (13047) (00083*)	50-100	good-luxuriant	>=50%	colourless without green or blue green colour
<i>Staphylococcus aureus subsp aureus</i> (25923) (00034*)	>=10 ³	inhibited	0%	
<i>Staphylococcus aureus subsp aureus</i> (6538) (00032*)	>=10 ³	inhibited	0%	
Key: * Corresponding WDCM numbers #: Formerly known as <i>Enterobacter sakazakii</i>				

Storage and Shelf-life

Store between 2-8°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. 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).

References

1. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983.
2. International Organization for Standardization. Microbiology of the food chain- Horizontal method for the detection of *Cronobacter* spp. Draft ISO/ TS 22964, 20176 (E).
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. 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.