



Technical Data

HiCrome™ Cronobacter Isolation Agar(CCI Agar)

M20621

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 /TS 22964: 2017.

Composition**

Ingredients	Gms / Litre
Tryptone#	7.000
Yeast extract	3.000
Sodium chloride	5.000
Sodium deoxycholate	0.250
5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside	1.500
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 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 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 deoxycholate inhibits the accompanying gram-positive flora.

Key: *: Formerly known as *Enterobacter sakazakii*

Type of specimen

Food samples

Specimen Collection and Handling

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

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

M2062I: Cultural characteristics observed after an incubation at 41.5±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Cronobacter sakazakii</i> ATCC 29544 (00214*)	50-100	good-luxuriant	≥50%	blue-green
<i>Cronobacter muytjensii</i> ATCC 51329 (00213*)	50-100	good-luxuriant	≥50%	blue-green
<i>Enterobacter cloacae</i> ATCC 13047 (00083*)	50-100	good-luxuriant	≥50%	colourless without green or blue green colour
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 25923 (00034*)	≥10 ³	inhibited	0%	
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 6538 (00032*)	≥10 ³	inhibited	0%	

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

- Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983.
- 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.), 2015, 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.

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Disclaimer :

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