

ChromArt

CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR

Dehydrated culture medium and ready-to-use plates



CCI Agar: blue-green colonies: *Cronobacter sakazakii*; white colonies with grey or black centre: *Salmonella* Enteritidis

1 - INTENDED USE

For the detection (presence/absence) of *Cronobacter* spp. in samples from the food chain, according to ISO 22964.

2 - COMPOSITION

TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Tryptone	7.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
5-bromo-4-chloro-3-indolyl- α -D-glucopyranoside	0.15 g
Sodium deoxycholate	0.25 g
Ammonium iron (III) citrate	1.00 g
Sodium thiosulfate	1.00 g
Agar	14.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.¹ Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteremia and meningitis, with case fatality rates ranging from 40-80%.^{1,2} The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.^{1,3,4}

Chromogenic Cronobacter Isolation (CCI) Agar is a culture medium for the determination of the presence or absence of *Cronobacter* spp. in samples from the food supply chain, according to ISO 22964.⁵ It must be used combined with Cronobacter Screening Broth.

Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium chloride maintains the osmotic balance. The selective agent of the medium is sodium deoxycholate which inhibits the growth of Gram-positive bacteria. The presence of sodium thiosulfate/ammonium iron citrate indicator system allows the differentiation of thiosulfate reductase positive bacteria (e.g., *Proteus*, *Salmonella*) that produce colonies with a slight black center. The medium contains the chromogenic compound 5-bromo-4-chloro-3-indolyl- α -D-glucopyranoside, a substrate for the detection of α -glucosidase: *Cronobacter* spp. possess α -glucosidase, cleave the chromogenic substrate so grow with blue to blue-green colonies

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31.9 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C mix well and pour into sterile Petri dishes

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	straw colour, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark amber, clear
Final pH at 20-25°C	7.3 \pm 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Chromogenic Cronobacter Isolation (CCI) Agar	Dehydrated medium	4080302	500 g (15.6 L)
		4080301	100 g (3.1 L)
Chromogenic Cronobacter Isolation (CCI) Agar	Ready-to-use plates	548030	2 x 10 plates ϕ 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile inoculation needles and loops, incubator and laboratory equipment as required, sterile Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

Food products and ingredients intended for human consumption and the feeding of animals; environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.⁵

9 - TEST PROCEDURE

Prepare the test sample in accordance with the relevant part of ISO 6887 dealing with the product concerned.

Add 10 g or 10 mL of sample to 90 mL of Buffered Peptone Water (REF 401278). For inoculum above 10 g preheat the broth to 34-38°C.

Incubate the pre-enrichment broth at 36 \pm 2 °C for 18 \pm 2 hours.

Transfer 0.1 mL of pre-enriched broth into 10 mL of Cronobacter Screening Broth (REF 401355)

Incubate the enrichment broth tubes at 41.5 \pm 1 °C for 24 \pm 2 hours





Streak a loopful of enrichment broth (about 10 µL) on a plate of CCI Agar and incubate upside down at $41.5 \pm 1^\circ\text{C}$ for 24 ± 2 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Typical *Cronobacter* colonies are small to medium-sized (1 mm to 3 mm) and blue to blue-green in colour.

Colonies of non-typical Gram-negative bacteria may develop on CCI Agar with the following characteristics: white, with or without a grey or black or green centre; some naturally pigmented colonies of non-*Cronobacter* may appear yellow or red.

Perform the confirmation tests on the typical colonies as reported by ISO 22964.⁵

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.⁵

Control strains	Incubation T° / t / ATM	Expected results
<i>C. sakazakii</i> ATCC 29544	$41.5^\circ\text{C} \pm 1^\circ\text{C} / 24 \text{ h} \pm 2 \text{ h} / \text{A}$	Good growth, blue-green colonies
<i>C. muytjensis</i> ATCC 51329	$41.5^\circ\text{C} \pm 1^\circ\text{C} / 24 \text{ h} \pm 2 \text{ h} / \text{A}$	Good growth, blue-green colonies
<i>E. cloacae</i> ATCC 13047	$41.5^\circ\text{C} \pm 1^\circ\text{C} / 24 \text{ h} \pm 2 \text{ h} / \text{A}$	White colonies
<i>E. faecalis</i> ATCC 19433	$41.5^\circ\text{C} \pm 1^\circ\text{C} / 24 \text{ h} \pm 2 \text{ h} / \text{A}$	Inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use Chromogenic *Cronobacter* Isolation (CCI) Agar are tested for productivity, specificity and selectivity with incubation at 41.5°C for 24 hours, by comparing the results with a previously approved Reference Batch.

Productivity is tested by a semi-quantitative ecometric technique with the target strains *C. sakazakii* ATCC 29544, *C. sakazakii* wild strain CB CRO.10.4 and *C. muytjensis* ATCC 51329. The amount of growth and colonies characteristics are evaluated after incubation: target strains grow with blue-green colonies. The specificity characteristics are tested by semi-quantitative ecometric technique with the following strains: *E. cloacae* ATCC 13047 and *S. Typhimurium* ATCC 14028. The amount of growth and colonies characteristics are evaluated: *E. cloacae* grows with white colonies, while *S. Typhimurium* grows with grey centered colonies.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433 and *B. cereus* ATCC 14579. After incubation, the growth of non-target strains is totally inhibited.

13 - LIMITATIONS OF THE METHOD

- Cronobacter* may be present in low numbers in the samples, along with other *Enterobacteriaceae*, such as *E. cloacae*, which may interfere in the determination of the target microorganism.⁵
- The use of large sample sizes can compromise the recovery of stressed *Cronobacter* spp. when interfering microflora are present, such as probiotics⁵

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates

Upon receipt, store plates in their original pack at $+2^\circ\text{C} / +8^\circ\text{C}$ away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean





area at +2°C /+8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium














Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/flasks) and the applied storage conditions (temperature and packaging). According to ISO 22964⁵ the plated medium may be kept at 5°C ± 3 °C for up to 14 days.

16 - REFERENCES

1. Yan QQ, Condell O, Power K, Butler F, Tall BD, Fanning S. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium, J App Microbiol 2012; 113:1-15
2. Friedemann, M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections. Eur J Clin Microbiol Infect Dis 2009; 28:1297–1304.
3. Bowen AB, Braden CR. Invasive Enterobacter sakazakii disease in infants. Emerg Infect Dis 2006;12:1185–1189.
4. Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning, S (2010) Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. Foodborne Pathog Dis 2010; 7:339–350.
5. ISO 22964:2017 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.

TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature imitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	 For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/06

Note: minor typographical, grammatical, and formatting changes are not included in the revision history

