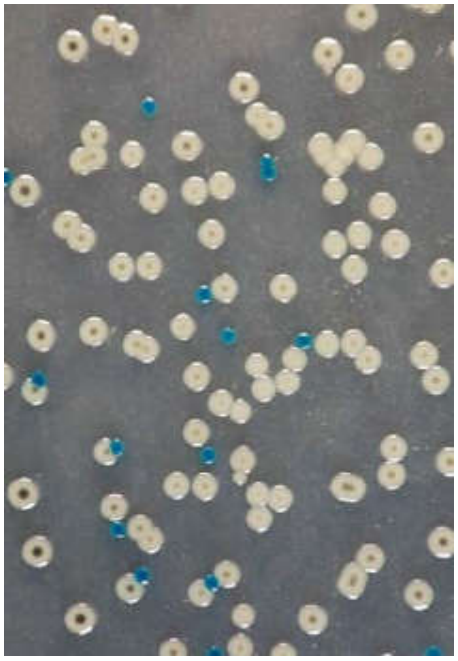
CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR



Chromogenic Cronobacter Isolation Agar: blue colonies: *Cronobacter sakazakii*; white colonies with gray center: *Salmonella enteritidis*

TYPICAL FORMULA (G/L)*

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

* The culture medium can be adjusted to adapt its performance to specifications

INTENDED USE

Chromogenic Cronobacter Isolation (CCI) Agar is a culture medium for the identification (presence/absence) of *Cronobacter spp* in samples from the food supply chain, according to the ISO 22964:2017. It must be used combined with Cronobacter Screening Broth.(401355)

PRINCIPLE OF THE METHOD

Cronobacter (*Enterobacter sakazakii*) is a Gram negative, rod-shaped bacterium, classified in the past as a yellow pigmented variant of *Enterobacter cloacae.*

It is a pathogen involved in meningitis, sepsis and necrotising enterocolitis in immature or immunocompromised newborns with high mortality rates. It is also responsible for nosocomial infections.

The diseases have been associated with the consumption of powdered milk or soy formulas for newborns, contaminated with *Cronobacter*. Episodes of infections have been reported in neonatal intensive care units in Canada, USA, UK, Greece, the Netherlands.

Chromogenic Cronobacter Isolation (CCI) Agar is a Gram negative selective medium containing a chromogenic compound for *Cronobacter* differentiation which grows with blue colonies.

The presence of sodium thiosulfate / iron ammonium citrate indicator system allows the differentiation of thiosulfate reductase positive bacteria (eg Proteus, Salmonella) that produce colonies with a slight black center.

PREPARATION METHOD

Dissolve 31,9 g in 1000 ml of cold purified water. Bring to boiling temperature under stirring and sterilize in an autoclave at 121°C for 15 minutes. Cool to 45-50°C, stir well and pour in plates.

CHEMICAL AND PHYSICAL CHARACTERISTICS

Aspect of the Dehydrated Culture media: fine powder straw colour.
Aspect of the plates: dark amber, clear.
Final pH of the medium: 7,3 ± 0,2

METHOD

Prepare the suspension of the sample according to the applicable ISO 6887 instructions.
Add 10 g or 10 ml of sample to 90 ml of Buffered Peptone Water (Casein) (REF 401278C). For inoculum above 10 g preheat the broth to 34-38 ° C. Incubate the pre-enrichment broth at 36 ± 2 ° C for 18 ± 2 hours.
Transfer 0.1 ml of pre-enriched broth incubated as in the previous step into 10 ml of Cronobacter Screening Broth.
Incubate the enrichment broth tubes at 41.5 ± 1 ° C for 24 hours ± 2 hours
Transfer a loopful of enrichment broth (about 10 ul) on a plate of CCI Agar and incubate upside down at 41.5 ± 1 ° C for 24 ± 2 hours. After incubation check the presence of typical *Cronobacter* colonies: 1 to 3 mm of blue or green blue color. Colonies of non-typical Gram negative bacteria may develop on CCI Agar with the following characteristics: white, with or without a gray or black or green center.
Perform the confirmation tests on the typical colonies as reported in the cited standard.

QUALITY CONTROL

It is responsibility of the user to perform quality control tests in accordance with the regulations and according to his own Laboratory experience. The following table shows some useful strains for the quality controls according to ISO 22964.

CONTROL STRAINS	INCUBATION	EXPECTED RESULTS
C.sakazakii ATCC 29544	41,5 °C ± 1 °C - 24 h ± 2 h.	Good growth, green-blue colonies
C.muytjensis ATCC 51329	41,5 °C ± 1 °C - 24 h ± 2 h.	Good growth, green-blue colonies
E.faecalis ATCC 19433	41,5 °C ± 1 °C - 24 h ± 2 h.	Inhibited
E cloacae ATCC 13047	41,5 °C ± 1 °C -24 h ± 2 h.	White colonies

LIMITS

- *Cronobacter* may be present in low numbers in the samples, along with other Enterobacteria, such as *E.cloacae*, which may interfere in the determination of the target microorganism.
- The use of large samples may compromise the determination of stressed *Cronobacter*, when there is a concomitant presence of non-target microflora (eg probiotics).

STORAGE

Store at 2-8°C, in a dry and dark place.
When stored as directed, the product remain stable until the expiry date shown on the label. Do not use beyond stated expiry date. Discard if there are any obvious signs of deterioration (color changes, hardening etc.).

PRECAUTIONS

The medium described here is not classified as dangerous according to current legislation.
As for all dehydrated media, the handling of this product must also be carried out with adequate protection of the respiratory tract.

REFERENCES

• ISO 22964/2017 Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

PRODUCTS

Description	Type	Cat. N°	Package
CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR	Dehydrated Culture Media	4080301 4080302	100 g (3.1 L) 500 g (15.7 L)
CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR	Ready to use 90 mm Plates	548030	20 plates

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