ThermoFisher SCIENTIFIC

Food testing

Validated detection and identification of *Cronobacter* species in 3 days

Thermo Scientific Cronobacter Precis Method

thermo scientific

Reinventing the standard for culture-based *Cronobacter* testing

Cronobacter (formerly *Enterobacter sakazakii*) are Gram-negative rod-shaped members of the family *Enterobacteriaceae* that have been implicated as causative agents of disease in premature infants (neonates), causing sepsis, meningitis, and necrotizing enterocolitis.

In particular, *Cronobacter* spp. have been isolated at low levels from powdered infant formulae (PIF), and the organisms' high tolerance to desiccation provides a competitive advantage in the dry environments of milk powder factories, increasing the risk of product contamination.

The Thermo Scientific™ Cronobacter Precis™ Method offers an alternative to the ISO 22964:2017 reference workflow by requiring only one enrichment prior to plate inoculation, significantly shortening overall time-to-result. This simplified and rapid workflow has been validated according to the ISO 16140-2:2016 standard by MicroVal™.

The Cronobacter Precis Method has been designed to overcome sensitivity and specificity issues, without the need to carry out non-standard handling steps, generating results in under three days.

Thermo Scientific™ Oxoid™ *Brilliance*™ Chromogenic Cronobacter Isolation (CCI) Agar plates may also be used as part of the ISO 22964:2017 horizontal method for the detection of *Cronobacter* spp. in food, animal feed and environmental samples.

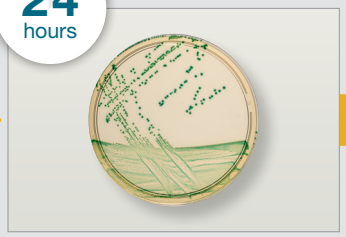
Samples of up to 375 g are diluted in the specified media for the matrix of interest and can be used for quality indicator testing prior to the addition of selective supplement.

For visually confirmed selective enrichment of PIF, infant cereals, and related ingredients (with probiotics), Thermo Scientific™ Oxoid™ PrecisBlue Supplement is added to the diluted sample (alternatively, a novobiocin formulation can be used).

16-26 hours

Samples are incubated overnight in a single, optimized enrichment medium at 34-38°C.

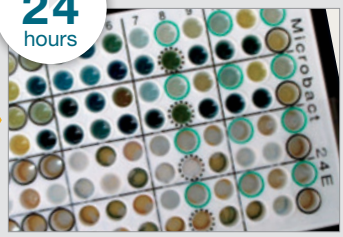
24 hours



A single *Brilliance* CCI Agar plate is inoculated using a 10 µL loop, before incubating overnight.

Blue-green colonies are presumptive-positive for *Cronobacter* species.

from 24 hours



Confirm presumptive-positive colonies with a choice of biochemical gallery (Thermo Scientific™ Oxoid™ Microbact™ GNB 24E or equivalent), PCR test (Thermo Scientific™ SureTect™ Cronobacter species PCR Assay) or ISO 16140-6 validated method.

Key Benefits:

- Simple procedure—no specialized equipment required
- Single overnight enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Option to incubate broths and plates at the same temperature for workflow simplicity
- Harmonized enrichment for *Cronobacter* and *Salmonella* for relevant matrices
- Reduced time to result: Under three days compared with up to five days for standard culture methods
- *Brilliance* CCI Agar improves recovery of *Cronobacter* by reducing background flora. Chromogens aid easy identification and differentiation by producing brightly colored colonies
- The Oxoid Microbact GNB 24E Kit or the SureTect Cronobacter species PCR Assay ensure a rapid and simple confirmation for isolated characteristic colonies from the CCI Agar plate
- For visually confirmed selective enrichment of PIF, infant cereals, and related ingredients (with probiotics), PrecisBlue Supplement is added to the diluted sample (alternatively, a novobiocin formulation can be used). Inert blue dye indicates supplemented buffered peptone water (BPW)

A rapid, simple, and robust culture-based workflow

The Cronobacter Precis Method has been validated and certified according to the ISO 16140-2:2016 standard by MicroVal™.

For more information about the MicroVal certification, see certificate 2020LR93 available at http://www.microval.org or obtain from our technical support team.

For flexibility, either the Microbact GNB 24E Kit or the SureTect Cronobacter species PCR Assay can be used for fast and simple confirmation of isolated characteristic colonies from the *Brilliance* CCI Agar plate.

However, depending on the legislation territory, it is also possible to use any appropriate EN ISO 16140-6:2019 validated confirmation method or any appropriate ISO 22964:2017 reference confirmation procedure.

Day 0

Infant formula with and without probiotics[1]:	Infant formula with or without probiotics, infant cereals with or without probiotics, and related ingredients[2]:		Production environmental samples[3]:	Production environmental samples:
10 g sample	Up to 375 g sample	Up to 375 g sample	25 g sample	25 g sample
10 g + 90 mL BPW	375 g + 3,375 mL BPW + vancomycin (6 mg/L)	375 g + 1,875 mL prewarmed BPW (with 6 mg/L novobiocin or PrecisBlue Supplement for probiotics)	25 g + 225 mL BPW + vancomycin (6 mg/L) Swab + 10 mL BPW + vancomycin (6 mg/L) Sponge + 100 mL BPW + vancomycin (6 mg/L)	25 g + 225 mL BPW Swab + 10 mL BPW Sponge + 100 mL BPW
34–38 °C for 16–22 h	34–38 °C for 20–26 h	34–38 °C for 18–24 h	34–38 °C for 18–24 h	34–38 °C for 20–26 h

Possibility to store for 72 h at 5±3 °C except for infant formula with probiotics (10 g sample size)

Day 1

Streak 10 µL onto *Brilliance* CCI Agar

24±2 h at 37±1 °C or 41.5±1 °C

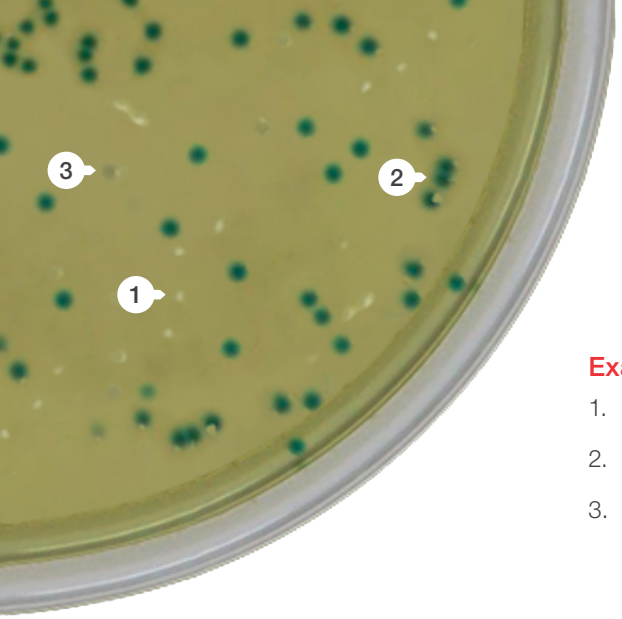
Possibility to store for 72 h at 5±3 °C

Day 2

Typical colonies (blue green), confirmed by one of the following:

Option 1: Biochemical galleries [Microbact GNB 24E Kit or equivalent]
Option 2: Tests of the ISO 22964 method
Option 3: Molecular hybridization test as described in EN ISO 7218L2007 (e.g. SureTect Cronobacter species PCR Assay)
Option 4: Appropriate ISO 16140-6:2019 validated confirmation method

1. No addition of α-amylase required.
2. No addition of α-amylase required. It is possible to test smaller sample sizes by adjusting the volume of BPW + vancomycin (6 mg/L) smaller sample size by adjusting the enrichment broth volume, maintaining the same dilution ratio.
3. For sampleing after cleaning process, premoisten: 1 swab + 1mL broth universal neutralizing (+9 mL BPW) OR 1 sponge + 10 mL broth universal neutralizing (+90 mL BPW)



Example results – Mixed culture

1. *Escherichia coli*
2. *Cronobacter* spp.
3. *Enterobacter cloacae*

Ordering information

Product description			Format	Order code
Oxoid Culture Media	Buffered Peptone Water	Buffered Peptone Water (ISO)	500 g, makes 25 L	CM1049B
		Buffered Peptone Water (ISO-meat peptone)	500 g, makes 25 L	CM1211B
	Novobiocin Supplement	Novobiocin Supplement - freeze-dried	10 vials of 10 mg	SR0181E
		Novobiocin Supplement - liquid (40 mL/vial)	10 vials of 40 mg	SR0249A
	PrecisBlue Supplement		1 vial makes 18 L	SR0259A
	Vancomycin Supplement	Vancomycin Supplement - freeze-dried	10 vials of 3 mg	SR0186E
		Vancomycin Supplement - freeze-dried	10 vials of 5 mg	SR0247E
Chromogenic Cronobacter Isolation Agar		CCI Agar – dehydrated	500 g makes 16.3 L	CM1122B
Microbact GNB Kit		Microbact GNB 24E Kit	40 tests/kit	MB1131A
SureTect Cronobacter species PCR Assay			1 kit	A56845

Please note that a range of alternative formats of culture media, such as Bagged Enrichment Media and Prepared Plate Media, are available.
Please contact your local representative or technical services to find out more.

For more information about the Thermo Scientific Cronobacter Precis Method and other rapid culture media methods for detecting foodborne pathogens **thermofisher.com/precis**