TECHNICAL DATA SHEET

CHROMOGENIC CRONOBACTER ISOLATION AGAR

DETECTION OF *CRONOBACTER*

1 INTENDED USE

CCI Agar is used for the detection of *Cronobacter* spp. in food products and ingredients intended for human consumption and the feeding of animals. It is also used for the control of production environmental samples.
CCI Agar is used in particular for the detection of *Cronobacter sakazakii* and spp. in milk powder, dehydrated products and their components found in infant foods.
The type composition of the Chromogenic *Cronobacter* Isolation Agar conforms to the formulation found in the directive NF EN ISO 22964.

2 HISTORY

Cronobacter sakazakii (formerly *Enterobacter sakazakii*) is a Gram negative bacillus, mobile, non-sporulated facultative anaerobe which forms pigmented yellow colonies after 48-72 hours of incubation on non-selective media. An opportunistic pathogen, it is notably at the origin of meningitis and enteritis, particularly with newborns and young children, and although the frequency is rather low at 1 in 100000, the mortality is high at roughly 20 to 50%. While the strains have been isolated from different food products, only those products destined for infant or baby foods are implicated in the infectious episodes.

Studies have shown that 100% of the *Cronobacter sakazakii* were positive for α-glucosidase when at the same time 100% of other species of *Enterobacter* were negative for this enzyme. On the basis of these observations, the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside (X-α-glucoside) has been proposed for differentiating this strains from other members of the *Enterobacteriacae* family.

3 PRINCIPLES

Tryptone stimulates the growth of *Cronobacter*.

Yeast extract is a source of complex vitamin B.

Sodium chloride maintains osmotic pressure.

Sodium desoxycholate allows the inhibition of Gram positive flora.

The enzyme α-glucosidase hydrolyzes the X-α-glucoside and liberates the aglycone 5 bromo-4-chloro-indolol. In the presence of oxygen, this aglycone is dimerized and forms the pigment bromo-chloro-indigo.

The association of ferric ammonium citrate and sodium thiosulfate allows the differentiation of enterobacteria that are H_2S positive from those of *Cronobacter*.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to achieve optimal performance.

For 1 liter of media:

- Tryptone ..... 7.00 g
- Yeast extract ..... 3.00 g
- Sodium choride ..... 5.00 g
- Sodium desoxycholate ..... 0.25 g
- Ammonium iron citrate ..... 1.00 g
- Sodium thiosulfate..... 1.00 g
- 5-bromo-4-chloro-3-indolyl,α-D-glucopyranoside ..... 150.0 mg
- Bacteriological agar..... 14.2 g

pH of the ready-to-use media at 25 °C: 7.3 ± 0.2.

5 PREPARATION

- Dissolve 31,6 g of dehydrated media (BK200) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, with constant agitation until complete dissolution.
- Dispense in tubes or in vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 47-50 °C.
- Pour into Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.

✓ **Reconstitution:**
31,6 g/L

✓ **Sterilization:**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

Detection protocol for *Cronobacter* according to NF EN ISO 22964 :

- Aseptically add 10 g or 10 mL of the product to test to 90 mL of Buffered Peptone Water.
- Use a mechanical mixer or stomacher if needed.
- Incubate the broth at 36 ± 2 °C for **18 ± 2 hours**.
- Re-isolate 0,1 mL of this pre-enrichment into 10 mL of screening broth for *Cronobacter* (BM155).
- Incubate at 41,5 ± 1,0 °C for 24 ± 2 hours.
- Streak for isolation on the surface of CCI agar (BM154) that has been brought to room temperature, with 0,1 mL of the enrichment media.
- Incubate at 41,5 ± 1,0 °C for 24 ± 2 hours.

✓ **Pre-enrichment:**
1 : 10 dilution
18 h at 36 °C

✓ **Enrichment:**
0.1 mL
24 h at 41.5 °C

✓ **Detection:**
Streak for isolation, 1 loop
24 h at 41.5 °C

Notes:

For larger sample sizes, preheat the Buffered Peptone Water to 36 ± 2 °C.
A concentration of trials may compromise the recuperation of stressed *Cronobacter* strains, in the presence of high background microflora. The user must determine the conformity of his/her operating protocol.

7 RESULTS

Colony aspect is as follows:

Microorganisms	Colony characteristics
Cronobacter spp.	Blue to blue-green colonies of 1 to 3 mm
Escherichia coli	White colonies with a greenish center
Salmonella spp, Proteus	Colonies with a black center
Gram positive bacteria	Inhibited

Proceed with the confirmation tests as described in the project Directive.

8 QUALITY CONTROL

Dehydrated medium: Beige, free-flowing and homogenous powder
Prepared media: Transparent, amber agar

Typical culture response after 24 of incubation at 41,5 °C:

Microorganisms		Growth	Characteristics
Cronobacter sakazakii	WDCM 00214	Good, score 2	Blue-green colonies
Cronobacter muytjensii	WDCM 00213	Good, score 2	Blue-green colonies
Enterobacter cloacae	WDCM 00083	Good, score 1-2	White colonies
Staphylococcus aureus	WDCM 00034	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated medium: 2-30 °C.
Pre-poured media in Petri dishes: 2-8 °C.
The expiration date is indicated on the label.

Prepared media in plates (*): 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated medium:
500 g vial..... BK200HA

Pre-poured media in Petri dishes (Ø 90 mm):
20 plates BM15408

11 BIBLIOGRAPHY

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Gurtler, J.B., Kornacki, J.L., and Beuchat, L.R.. 2005. *Enterobacter sakazakii*: a coliform of increased concern to infant health. International Journal of Food Microbiology, 104 : 1-34.

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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