TECHNICAL DATA SHEET

COMPASS® *ENTEROBACTER SAKAZAKII* AGAR

DETECTION OF *CRONOBACTER SAKAZAKII*

1 INTENDED USE

The COMPASS® *Enterobacter sakazakii* Agar is used 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 project directive PR 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.

The choice of the incubation temperature fixed at 44 °C, plus the association of sodium desoxycholate and crystal violet combines to inhibit the growth of a large spectrum of contaminating microflora.

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.

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 chloride ..... 5,00 g
- Sodium desoxycholate ..... 0,60 g
- Cristal violet ..... 2,0 mg
- 5-bromo-4-chloro-3-indolyl,α-D-glucopyranoside ..... 150,0 mg
- Bacteriological agar ..... 14,40 g

pH of the ready-to-use media at 25 °C : 7,0 ± 0,2.

5 PREPARATION

- Dissolve 30,2 g of dehydrated media (BK188) in 1 liter of distilled or demineralized water.
- Slowly stir until complete dissolution.
- Divide into vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold surface.

✓ **Reconstitution : 30,2 g/L**

✓ **Sterilization : 15 min at 121 °C**

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator with the covers partially removed.
- On the surface of plates prepared as above, or on pre-poured plates (BM120) brought to room temperature, inoculate by streaking a loop of enrichment media (mLST broth, BM121).
- Incubate at 44 ± 1°C for 24 ± 2 hours.

✓ **Inoculation : a loop of enrichment media**

✓ **Incubation : 24 h at 44°C**

7 RESULTS

The aspect of the colonies are as follows :

Microorganisms	Colony characteristics
Cronobacter sakazakii	Blue-violet colonies
Escherichia coli	Grey to violet colonies
Enterobacter spp., *Klebsiella* spp.	Grey to violet colonies
Gram positive bacteria	Inhibited

See ANNEX 1 : PHOTO SUPPORT.

Note : The strains of *Cronobacter* present generally a characteristic pigmentation blue-green. However, it is possible that some strains weakly α-glucosidase-positives could be confused with certain non-targeted Gram-negative microorganisms, which by incorporating the crystal violet, present a similar aspect.

8 QUALITY CONTROL

Dehydrated media : beige powder, free-flowing and homogeneous.
Prepared media : violet agar.

Typical culture response after 24 of incubation at 44 °C:

Microorganisms		Growth	Characteristics
Cronobacter sakazakii	WDCM 00214	Good, score 2	Blue-green colonies
Cronobacter sakazakii	CIP 104951	Good, score 2	Blue-green colonies
Escherichia coli	WDCM 00013	Good, score 2	Violet colonies
Enterobacter cloacae	WDCM 00083	Good, score 2	Violet colonies
Staphylococcus aureus	WDCM 00034	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

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

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

10 PACKAGING

Dehydrated media :
500 g bottle BK188HA

Pre-poured media in Petri plates (Ø 90 mm) :
20 plates BM12008

11 BIBLIOGRAPHY

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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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ANNEX 1 : PHOTO SUPPORT

COMPASS® *Enterobacter sakazakii* Agar

Detection of *Cronobacter sakazakii*.

Results :

Growth obtained after 24 hours of incubation at 44 °C.