

Cronobacter Selective Broth (NCM0227)

Intended Use

Cronobacter Selective Broth (CSB) is recommended by ISO 22964 for the isolation of *Cronobacter* spp. (formerly *Enterobacter sakazakii*), and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Although rarely causing infections in immunocompetent adults, members of the *Cronobacter* genus have been implicated in sepsis, meningitis and necrotizing enterocolitis with a high death rate in neonates. These opportunistic pathogens are common in the environment and their ability to survive desiccation presents a significant risk for post-pasteurization contamination and survival in spray dried milk products. Enzymatic digest of animal tissues and meat extract provide a source of nitrogen and essential vitamins. High levels of sodium chloride and sucrose, the addition of vancomycin and an elevated incubation temperature produce a selective and specific medium. *Cronobacter* spp. ferment the sucrose in this broth, resulting in a decrease in pH and thus a change of color from purple to yellow or red due to the presence of the pH indicator bromocresol purple. Most other organisms do not grow in this medium, while *E. coli* are suppressed, resulting in a turbid medium with no changes in color. According to ISO 22964:2017, subculture is performed after pre-enrichment in Buffered Peptone Water (BPW).

Typical Formulation

Enzymatic Digest of Animal Tissues	10.0 g/L
Meat Extract	3.0 g/L
Sodium Chloride	5.0 g/L
Bromocresol Purple	0.04 g/L
Sucrose	10.0 g/L

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

NCM4004 Vancomycin (10mg/L)

Precaution

Refer to SDS

Preparation

1. Disperse 28 grams of the medium in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium if necessary.
3. Autoclave at 121 °C for 15 minutes.
4. Cool to ambient temperature, then add 2 vials of NCM4004-0.5* Vancomycin (10mg/L) supplement, each reconstituted using 5mL of sterile deionized/RO water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

Test Procedure

- According to ISO 22964:2017, a test portion of 10 g or 10 mL is added to BPW ISO (NCM0015) and incubated 18 h at 34-38 °C.



Technical Specification Sheet



- After incubation, 0.1 mL of the pre-enriched BPW is added to 10 mL of CSB, which is then incubated at $41.5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $24 \pm 2\text{ h}$.
- Sub-culture onto *Cronobacter* Chromogenic Isolation Agar (NCM1008) for further isolation and identification.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and yellow to pale green.

Prepared Appearance: Prepared medium is a clear, purple liquid.

Minimum QC:

Cronobacter sakazakii WDCM 00214 Turbidity, colour change to yellow or orange
+ *Staphylococcus aureus* WDCM 00032 or >10 characteristic colonies on CCI
WDCM 00034

Cronobacter muytjensii WDCM 00213 Turbidity, colour change to yellow or orange
+ *Staphylococcus aureus* WDCM 00032 or >10 characteristic colonies on CCI
WDCM 00034

Staphylococcus aureus WDCM 00032 or Total or partial inhibition, no colour change
WDCM 00034 ≤100 colonies on TSA

Results

Growth Characteristics		
Organism	Growth	Color
<i>Cronobacter</i> spp.	Turbid growth	Yellow/Orange
<i>Escherichia coli</i>	Turbid growth	Purple
<i>Staphylococcus aureus</i>	No growth	N/A

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 22964:2017 Microbiology of the food chain – Horizontal method for the detection of *Cronobacter* spp.
2. Iversen, C. (2008). Development of a novel screening method for the isolation of "*Cronobacter*" spp. (*Enterobacter sakazakii*). Appl. Environ. Microbiol., 74(8), 2550-2553.



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