

Cronobacter Selective Broth (CSB) ISO

Cat. 2143

For the selective enrichment of Cronobacter spp. from food products and environmental samples.

Practical information

Applications	Categories
Selective isolation	Cronobacter

Industry: Food

Regulations: ISO 22964



Principles and uses

Cronobacter Selective Broth (CSB) is a selective medium for the enrichment of Cronobacter spp. from food products and ingredients intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. The ISO normative 22964 recommends this medium for the selective enrichment of Cronobacter spp.

Enzymatic digest of animal tissues and meat extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sucrose is the fermentable carbohydrate providing carbon and energy. Bromocresol purple is a pH indicator.

Cronobacter (formerly Enterobacter sakazakii) is currently considered an emerging pathogen responsible for, un-weaned babies, risking severe meningitis and necrotic enterocolitis that can be the cause of mortality rate between 40-80%.

The pathogenicity of Cronobacter for un-weaned babies' makes it necessary to review the manufacturing process of the milk-based products specialized for babies, guaranteeing the absence of the bacteria in the final product. Additional prevention measures at a hospital include the sanitary hygiene of the prepared food and reducing the time between the preparation and its administration, to impede the multiplication of microorganisms.

ISO 22964 FDIS:2016 describes a horizontal method for the detection of Cronobacter spp. and recommend this medium for the enrichment of Cronobacter spp. in food, in animal feed and in environmental samples. A yellow/orange coloration in the broth after incubation (due to sucrose fermentation) indicates presumptive Cronobacter. If the broth is still purple in colour after incubation, the sample is presumed to be negative for Cronobacter.

Formula in g/L

Bromocresol purple	0,04	Beef extract	3
Sodium chloride	5	Sucrose	10
Enzymatic digest of animal tissues	10		

Preparation

Suspend 28,04 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 50 °C and aseptically add 2 vials of Vancomycin Supplement (Cat. 6072) so as to obtain a final vancomycin concentration of 10 mg per litre of CSB. Homogenize gently and dispense into tubes in amounts of 10 ml.

Instructions for use

For the detection of Cronobacter spp. according to ISO 22964:

- Prepare the test portion by adding 10 g or 10 ml of the sample to 90 ml of a previous BPW enrichment medium.
- Incubate at 34-38 °C for 18±2 hours.

- Transfer 0,1 ml of the obtained culture to 10 ml of CSB and mix well.
- Incubate at 41,5±1 °C for 24±2 hours.
- Using the previous enrichment culture, inoculate the surface of an CCI Agar (Cat. 1446) and incubate at 41,5±1 °C for 24±2 hours.
- Confirmation.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Straw colored	Purple	7,4±0,2

Microbiological test

Incubation conditions: (41,5±1 °C / 24±2 h).

Inoculation conditions: Productivity Qualitative (<100 CFU) / Selectivity Qualitative (10⁴-10⁶ CFU).

Microorganisms	Specification	Characteristic reaction
Cronobacter sakazakii ATCC 29544 + Staphylococcus aureus ATCC 25923	Color change of CSB >100 colonies on CCI	Yellow color of CBS; small to medium-size (1-3 mm) blue to blue-green colonies on CCI
Cronobacter muytjensii ATCC 51329 + Staphylococcus aureus ATCC 25923	Color change of CSB >100 colonies on CCI	Yellow color of CBS; small to medium-size (1-3 mm) blue to blue-green colonies on CCI
Cronobacter sakazakii ATCC 29544 + Staphylococcus aureus ATCC 6538	Color change of CSB >100 colonies on CCI	Yellow color of CBS; small to medium-size (1-3 mm) blue to blue-green colonies on CCI
Staphylococcus aureus ATCC 25923	Total o partial inhibition on TSA <100 colonies	Purple color of CSB
Cronobacter muytjensii ATCC 51329 + Staphylococcus aureus ATCC 6538	Color change of CSB >100 colonies on CCI	Yellow color of CBS; small to medium-size (1-3 mm) blue to blue-green colonies on CCI
Staphylococcus aureus ATCC 6538	Total o partial inhibition on TSA <100 colonies	Purple color of CSB

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

ISO normative 22964:2016 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.
GUILLAUME-Gentil, O., Sonnard, V. Kandahai, M.C., Mauragg, J.D. and Jootsen, H. A simple and Rapad Cultural Method for Detection of Enterobacter Sakazakii in environmental samples. Journal of Food. Protection, 68 (1), 2005, pp. 64-69.