

**Also known as**

ESIA

**Specification**

A solid medium, selective and differential for the presumptive isolation of *Cronobacter sakazakii* (*E.sakazakii*) in samples of milk and dairy products, according to ISO / TS 22964 and IDF / RM 210.

**Formula \* in g/L**

Pancreatic casein peptone.....	7,000
Yeast extract.....	3,000
Sodium chloride.....	5,000
Sodium deoxycholate.....	0,600
5-Br-4-Cl-3-indolyl- $\alpha$ -D-glucopyranoside.....	0,150
Cristal violet .....	0,002
Agar.....	15,000

 Final pH 7,0  $\pm$  0,2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

**Directions**

Suspend 30.75 g of the powder in 1 liter of distilled water. Bring to a boil until completely dissolved. Distribute into suitable containers and sterilize by autoclaving at 121 ° C for 15 minutes.

**Description**

*Cronobacter* spp (formerly *Enterobacter sakazakii*) can cause various clinical conditions such as necrotizing enterocolitis, bacteremia, and even meningitis. These infections can be fatal in newborns and even if they survive meningitis, neurological damage can occur throughout life. To reduce the risk of infection *via* digestive tract through the infant nutrient preparations based on milk, a regulation on *Cronobacter* detection in milk and dairy products has been published jointly developed by ISO and FIL-IDF in which the culture medium for the presumptive identification is Sakazakii Chromogenic Agar.

*Cronobacter* produces  $\beta$ -glucosidase, which hydrolyses 5-bromo-4-chloro-3-indolyl- $\beta$ -glucopyranoside and releases the colored fraction of the substrate. That results in blue-green colonies, which allow differentiating these bacteria from other *Enterobacteriaceae* present in the sample. Deoxycholate and crystal violet in the culture medium inhibits the growth of gram-positive bacteria

**Technique**

The detailed work methodology can be found in ISO / TS 22964:2006 and IDF / RM 210:2006 which refers to the technician concerned.

In summary, the recommended method is a pre-enrichment in BPW à 37 ° C, a selective enrichment in modified lauryl tryptose with vancomycin broth à 44 ° C and presumptive isolation on chromogenic agar à 44 ° C.

All suspect colonies should be confirmed subsequently by established methods, serological, biochemical or genetics.

Limitations:

The former species *Enterobacter sakazakii* has now become the new genus *Cronobacter* with seven described species, whose behavior and colonial aspects may vary depending on growing conditions.

Some strains of these species can not grow or grow very poorly à temperatures of 44 ° C and above.

It is strongly recommended that the final identification is made with supporting evidence.

**Quality control**
**Incubation temperature:** 44° C  $\pm$  1,0

**Incubation time:** 24 h  $\pm$  2

**Inoculum:** Practical range 100  $\pm$  20 CFU. Min. 50 CFU (Productivity) / 10<sup>4</sup>-10<sup>6</sup> CFU (Selectivity) /  $\geq$  10<sup>3</sup> CFU (specificity) according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
<i>Cronobacter sakazakii</i> ATCC® 29544	Good	Blue-green colonies of 1-2 mm diameter
<i>Cronobacter muytjensii</i> ATCC® 51329	Good	Blue-green colonies of 1-2 mm diameter
<i>Escherichia coli</i> ATCC® 25922	Poor to good	Straw yellow colonies of 0.25-1 mm
<i>Staphylococcus aureus</i> ATCC® 25923	Fair to poor	Inhibited or no growth
<i>Enterobacter aerogenes</i> ATCC® 13048	Good	Colorless colonies

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**References**

- FIL-IDF/RM 210 (2006) Lait et produits laitiers - Detection de l'Enterobacter sakazakii.
- FORSYTHE, S.J. (2012) Myths and legends of Cronobacter: A new bacterial pathogen of babies? Microbiology Today 31:1:30-33
- HOCHÉL, I., H. RŮZICKOVÁ, I. KRÁSNÝ & H. DEMNEROVÁ (2012) Occurrence of Cronobacter spp. In retail foods. J. Appl Microbiol 112:6:1257-1265.
- ISO / TS 22964 (2006) Milk and milk products. - Detection of Enterobacter sakazakii
- IVERSEN, C. & S.J. FORSYTHE (2006) Comparison of media for the isolation of Enterobacter sakazakii. Appl. Environ. Microbiol 73:1:48-52.
- JOSEPH, S. & S. J. FORSYTHE (2011) Association of Cronobacter sakazakii ST4 with neonatal infections. Emerging Infectious Disease 17:1713-1715.

**Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).