ChromArt

ENTEROBACTER SAKAZAKII ISOLATION AGAR (ESIA)

Dehydrated culture medium and ready-to use plates

ESIA: blue-green colonies: *Cronobacter sakazakii*; white colonies: *Salmonella* Enteritidis.

1 - Intended use

Chromogenic selective medium for the detection of *Cronobacter (Enterobacter) sakazakii* in foods.

2 - Composition

Typical formula after reconstitution with 1 L of water*

Dehydrated medium and ready to use plates

Peptone	7.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
Sodium deoxycholate	0.60 g
Crystal violet	0.002 g
X α-glucoside	0.150 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - Principle of the method and explanation of the procedure

Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1] Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteremia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]

Enterobacter Sakazakii Isolation Agar (ESIA) is a selective medium for Gram-negative bacteria, containing a chromogenic compound for the differentiation of *Cronobacter sakazakii* that cultivates with blue-green colonies.

The use of Buffered Peptone Water as a non-selective enrichment, mLST Broth (401476) as a selective enrichment and the ESIA isolation medium allow the specific detection of *C.sakazakii* in food samples especially in milk powder and powdered infant formula. The ESIA medium and work procedure described below are in accordance with the withdrawn standard ISO/TS 22964:2006[4], replaced by ISO Standard 22964:2017.[5]

4- Directions for medium preparation

Suspend 30.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - Physical characteristics

Dehydrated medium appearance	pale violet, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale violet, clear
Final pH of complete medium (at 20-25°C)	7.0 ± 0.2

6 - Materials provided – packaging

Product	Type	REF	Pack
Enterobacter Sakazakii Isolation Agar (ESIA)	Dehydrated medium	4014782	500 g (16.2 L)
Enterobacter Sakazakii Isolation Agar (ESIA)	Ready to use plates	541478	2 x 10 plates ø 90 mm

7 - Materials required but not provided

Autoclave, water-bath, sterile inoculation needles and pipettes, incubator and laboratory equipment as required, sterile Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - Specimens

Milk powder, powdered infant formula and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - Test procedure

1. Prepare the initial sample suspension (primary dilution) by adding x g of the test sample to 9 times x ml of Buffered Peptone Water Casein (REF 401278C or 401278): e.g. 25 g + 225 mL of Buffered Peptone Water Casein or 10 g + 90 mL of Buffered Peptone Water Casein.
2. Incubate at 37 ± 1°C for 18 ± 2 hours.
3. After incubation of the inoculated pre-enrichment medium, transfer 0.1 ml of the obtained culture into 10 mL mLST Broth (REF 401476).
4. Incubate at 44 ± 0.5°C for 24 ± 2 hours.
5. After incubation, streak a 10 µl loopful from the mLST broth onto the surface of the ESIA plate and incubate at 44 ± 1°C for 24 ± 2 hours.

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10 - READING AND INTERPRETATION

After incubation, observe bacterial growth, recording each specific morphological and chromatic characteristic of the colonies.
Presumptive positive result for *C. sakasakii*: presence of blue to green colonies, 1 to 3 mm in diameter.
Negative result for *C. sakasakii*: absence of typical blue-green colonies or presence of mauve-violet colonies.
Confirm colonies with biochemical tests recommended by ISO 22964.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Productivity: *Cronobacter sakazakii* good growth, green to blue colonies
Specificity: *Enterobacter gergovieae* scanty growth, mauve colonies
Selectivity: *S. aureus*, inhibited
Incubation at 44°C for 24 hours.

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale representative samples of all lots of dehydrated and ready-to-use ESIA are tested for productivity, specificity and selectivity, by comparing the results with a previously approved Reference Batch.
The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *C.sakazakii* CB CRO10.4, *C. muytjensii* ATCC 51329. After incubation at 44°C for 24 hours the target strains exhibit good growth with blue-green colonies.
Specificity is assessed by modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E.gergoviae* CB AES2.1strain. After incubation at 44°C for 24 hours the strain exhibits scanty growth with mauve/purple colonies.
The selectivity is tested with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *E.coli* ATCC 25922, *S.aureus* ATCC 25923, *B.cereus* ATCC 14579. The growth of the non-target strains is totally inhibited.
CB: Biolife microbial collection.

13 - LIMITATIONS OF THE METHOD

- Some coliforms grow on ESIA with violet colonies, easily distinguishable from the blue colonies of *C. sakazakii*.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the ready-to-use plates are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates
Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).
Dehydrated medium
Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 22964:2006, the self-prepared plates may be kept at 0 °C to 5 °C for up to 14 days.[4]

16 - REFERENCE

1. Carol Iversen et al., The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies BMC Evol Biol. 2007; 7: 64.
2. Simmons BP et al. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of powdered infant formula. Infect Control Hosp Epidemiol 1989; 10: 398.
3. Van Acker J et al. Outbreack of necrotizing enterocolitis associated with *E.sakazakii* in powdered milk formula. J Clin Microbiol 2001; 39:293-297.
4. ISO/TS 22964:2006. Milk and milk products – Detection of *Enterobacter sakazakii*
5. ISO 22964:2017. Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history

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