

**MODIFIED LAURYL SULFATE TRYPTOSE (mLST)  
BROTH BASE  
ENTEROBACTER SAKAZAKII ISOLATION AGAR (ESIA)**  
Enrichment selective broth and selective chromogenic plating medium for the detection of  
*Cronobacter (Enterobacter) sakazakii* in milk and milk products.

**TYPICAL FORMULATIONS**

**mLST BROTH BASE –****POWDERED MEDIUM (g/L)**

PEPTONE*	20.00
LACTOSE	5.00
POTASSIUM PHOSPHATE MONOBASIC	2.75
POTASSIUM PHOSPHATE BIBASIC	2.75
SODIUM LAURIL SULFATE	0.10
SODIUM CHLORIDE	34.00

\* Enzymatic digest of animal and plant tissue

**ESIA - POWDERED MEDIUM AND READY TO USE PLATES (g/L)**

PEPTONE	7.00
YEAST EXTRACT	3.00
SODIUM CHLORIDE	5.00
SODIUM DESOXYCHOLATE	0.60
CRYSTAL VIOLET	0.002
X- $\alpha$ -GLUCOSIDE	0.150
AGAR	15.00

**VANCOMYCIN ANTIMICROBIC SUPPLEMENT (x vial)**

VANCOMYCIN	25 mg
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**mLST BROTH – READY TO USE TUBES (g/L)**

PEPTONE*	20.00
LACTOSE	5.00
POTASSIUM PHOSPHATE MONOBASIC	2.75
POTASSIUM PHOSPHATE BIBASIC	2.75
SODIUM LAURIL SULFATE	0.10
SODIUM CHLORIDE	34.00
VANCOMYCIN	0.01

\* Enzymatic digest of animal and plant tissue

**METHOD OF PREPARATION****Modified Lauryl Sulfate Tryptose (mLST) Broth Base**

Suspend 64.6 g in 1000 ml of cold distilled water. Heat to dissolve and sterilize by autoclaving at 121 °C for 15 minutes. Cool to room temperature and add 2 ml of the vial contents of Vancomycin Antimicrobial Supplement (REF 4240057), reconstituted with 5 ml of sterile distilled water (the reconstituted supplement can be stored at -20 °C for 1 month). Mix well and distribute into sterile tubes (10 ml/tube)  
final pH 6.8  $\pm$  0.2

**Enterobacter Sakazakii Isolation Agar (ESIA)**

Suspend 30.8 g in 1000 ml of cold distilled water. Heat to boiling stirring constantly; sterilize by autoclaving at 121 °C for 15 minutes. Cool to 45-50 °C and distribute into sterile petri dishes.  
final pH 7.0  $\pm$  0.2

**TECHNIQUE**

- To prepare the primary dilution, add x g of the test sample to 9 times x ml of Buffered Peptone Water (REF 401278).
- Allow dry samples to disperse in the liquid without stirring. If a sample has not been dissolved completely after 30 min, than mix it gently with the medium.
- Incubate the inoculated Buffered Peptone Water at 37 °C  $\pm$  1 °C for 18 h  $\pm$  2 h.
- After incubation of the inoculated pre-enrichment medium, transfer 0,1 ml of the obtained culture into 10 ml of mLST/vancomycin medium.
- Incubate at 44 °C  $\pm$  0,5 °C for 24 h  $\pm$  2 h.

- It is recommended to use either a water bath (6.3) or a forced-air incubator to ensure that the maximum temperature (44,5 °C) is not exceeded.
- After incubation of the inoculated mLST Broth with vancomycin streak a loopful (ca. 10 µl) onto the surface of the ESIA plate.
- Incubate the plate at 44 °C ± 1 °C for 24 h ± 2 h.
- After incubation, examine the chromogenic plate for the presence of typical colonies of presumptive *Cronobacter (Enterobacter) sakazakii*.
- Typical colonies are small to medium sized (1 mm to 3 mm) green to blue-green colonies. Non-typical colonies are often slightly transparent and violet coloured.
- Confirm the colonies with the biochemical tests recommended by ISO/TS 22964.

**User Quality Control (mLST Broth)**Productivity: *C.sakazakii* good growthSelectivity: *S.aureus* ATCC 25923: no growth

Incubation at 44 °C ± 0,5 °C for 24 h ± 2 h

**User Quality Control (ESIA)**Productivity: *C.sakazakii* good growth, blue coloniesSpecificity: *Enterobacter gergovieae*: good growth, purple coloniesSelectivity: *S.aureus* ATCC 25923: no growth

Incubation at 44 °C ± 0,5 °C for 24 h ± 2 h

**STORAGE**

mLST Broth (powdered medium): 10-30 °C

ESIA (powdered medium): 2-8 °C

Ready to use tubes and plates: 2-8 °C

**LIMITS**

Some coliform bacteria grow on ESIA plates with violet colonies.

**REFERENCES**

- ISO/TS 22694:2006 Milk and milk products – Detection of *Enterobacter sakazakii*

**PACKAGING**

4014762	Modified Lauryl Sulfate Tryptose (mLST) Broth Base	500 g (7,8 L)
4014782	Enterobacter Sakazakii Isolation Agar (ESIA)	500 g (16,2 L)

**SUPPLEMENT**

4240057	Vancomycin Antimicrobial Supplement	10 VIALS
5514762	Modified Lauryl Sul fate Tryptose (mLST) Broth	20x10 ml (ready to use tubes)
541478	Enterobacter Sakazakii Isolation Agar (ESIA)	20 ready to use plates