

**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)**

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|-------------------------------|-------|
| 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)

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|------------------------|-------|
| PEPTONE | 7.00 |
| YEAST EXTRACT | 3.00 |
| SODIUM CHLORIDE | 5.00 |
| SODIUM DESOXYCHOLATE | 0.60 |
| CRYSTAL VIOLET | 0.002 |
| X- α -GLUCOSIDE | 0.150 |
| AGAR | 15.00 |

VANCOMYCIN ANTIMICROBIC SUPPLEMENT (x vial)

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|------------|-------|
| VANCOMYCIN | 25 mg |
|------------|-------|

mLST BROTH – READY TO USE TUBES (g/L)

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|-------------------------------|-------|
| 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 gergoviae*: 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 |