

## TECHNICAL DATA SHEET

# MODIFIED LAURYSULFATE-TRYPTOSE VANCOMYCINE BROTH (mLST/V)

SELECTIVE ENRICHMENT OF *CRONOBACTER SAKAZAKII*

## 1 INTENDED USE

Modified Laurylsulfate-Tryptose Vancomycin broth (mLST/V) is a selective enrichment medium used for the detection of *Cronobacter sakazakii* in milk and milk products, according to the standard ISO/TS 22964.

## 2 HISTORY

The base medium (without vancomycin) was formulated by Malmann & Darby who demonstrated in 1941 that among a large number of wetting agents, sodium laurylsulfate presented the best characteristics for a selective agent while not inhibiting coliforms. Lévine then demonstrated that the medium reduced the number of false positives by inhibiting sporeforming bacteria.

Through the addition of vancomycin and by an increase in the level of sodium chloride, the medium allows the specific detection of *Cronobacter sakazaki*.

## 3 PRINCIPLES

Sodium laurylsulfate, associated with vancomycin, in addition to the high level of sodium chloride, prevents the development of most contaminating secondary flora.

By virtue of its excellent fertility, as well as to the presence of buffering phosphates, the mLST/V allows the rapid development of *Cronobacter sakazakii* even in low numbers.

## 4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of medium :

- Tryptose .....	20,00 g
- Lactose.....	5,00 g
- Dipotassium phosphate.....	2,75 g
- Monopotassium phosphate.....	2,75 g
- Sodium chloride .....	34,00 g
- Sodium laurylsulfate .....	0,10 g
- Vancomycin.....	0,01 g

pH of the ready-to-use media at 25 °C : 6,8 ± 0,2.

## 5 PREPARATION

- Dissolve 64,6 g of dehydrated media (BK190) in 1 liter of distilled or demineralized water.
- Stir until complete dissolution.
- Divide into tubes, 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**  
64,6 g/L

✓ **Sterilization :**  
15 min at 121 °C

## 6 INSTRUCTIONS FOR USE

- Inoculate the tubes, prepared as above or use the ready-to-use tubes (BM121) with 0,1 mL of the pre-enrichment broth (Buffered Peptone water).
- Incubate for 24 ± 2 hours at 44,0 ± 1,0 °C.

✓ **Inoculation :**  
**0,1 mL**  
✓ **Incubation :**  
**24 h at 44 °C**

## 7 RESULTS

Presumptive positive tubes demonstrate cloudiness or turbidity.  
Re-inoculate a loop from each tube onto COMPASS *Cronobacter sakazakii* (BM120).

## 8 QUALITY CONTROL

**Dehydrated media :** dead white powder, free-flowing and homogeneous.

**Prepared media :** limp, clear amber solution.

Typical culture response after 24 hours of incubation at 44 °C, followed by subculture sur COMPASS® *Cronobacter sakazakii* Agar

Microorganisms		Growth
<i>Cronobacter sakazakii</i>	WDCM 00214	> 10 characteristic colonies (blue-green)
+ <i>Escherichia coli</i>	WDCM 00013	
+ <i>Proteus mirabilis</i>	WDCM 00023	
<i>Cronobacter sakazakii</i>	CIP 57.33	> 10 characteristic colonies (blue-green)
+ <i>Enterobacter cloacae</i>	WDCM 00083	
+ <i>Staphylococcus aureus</i>	WDCM 00034	
<i>Escherichia coli</i>	WDCM 00013	Grey to violet colonies
<i>Proteus mirabilis</i>	WDCM 00023	Grey to violet colonies
<i>Enterobacter cloacae</i>	WDCM 00083	Partially inhibited, score 0-1
<i>Staphylococcus aureus</i>	WDCM 00034	Inhibited, score 0

## 9 STORAGE / SHELF LIFE

**Dehydrated media :** 2-30 °C.

**Ready-to-use media in tubes :** 15-25 °C.

The expiration date is indicated on the label.

**Prepared media in tubes (\*) :** 180 days at 15-25 °C.

(\*)Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

The refrigeration of the tubes is not advised as they can develop cloudiness and precipitates that disappear after return to room temperature.

## 10 PACKAGING

**Dehydrated media :**

500 g bottle ..... BK190HA

**Ready-to-use media in tubes :**

50 x 10 mL tubes ..... BM12108

## 11 BIBLIOGRAPHY

Mallmann, W.L., and Darby, C.W.. 1941. Uses of a lauryl sulfate tryptose broth for the detection of coliform organisms. American Journal of Public Health and the Nations Health, **31** : 127-134.

ISO/TS 22964. Février 2006. Lait et produits laitiers. Détection de l'*Enterobacter sakazakii*.

## 12 ADDITIONAL INFORMATION

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The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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