TECHNICAL DATA SHEET

LAURYLSULFATE-TRYPTOSE BROTH

SELECTIVE ENRICHMENT OF ESCHERICHIA COLI AND OTHER COLIFORMS

1 INTENDED USE

Laurylsulfate-Tryptose broth is a selective enrichment media used for the detection and enumeration of *Escherichia coli* and coliforms in water and food products.
The medium was formulated by Malmann and Darby who showed, in 1941, that sodium laurylsulfate was the best selective agent among a number of surfactants and did not inhibit the growth of coliform bacteria. Levine later showed that the medium reduced the number of false positives by inhibiting the growth of sporulated gas-producing bacteria.

The typical composition responds to that defined in the standards NF T90-413 and NF ISO 7251.

2 PRINCIPLES

Sodium laurylsulfate considerably inhibits the development of accompanying microbial flora without inhibiting coliform species.

Because of its excellent nutritive capacity, as well as the presence of phosphate buffer, Laurylsulfate-Tryptose Broth enables coliform bacteria to rapidly develop and release large quantities of gas from the fermentation of lactose, even with small inocula.

3 TYPICAL COMPOSITION

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

For 1 liter of media (single strength):

- Tryptose ..... 20,00 g
- Lactose ..... 5,00 g
- Di-potassium phosphate ..... 2,75 g
- Potassium dihydrogen phosphate ..... 2,75 g
- Sodium chloride ..... 5,00 g
- Sodium lauryl sulfate ..... 0,10 g

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

4 PREPARATION

Single strength broth

- DIssolve 35,6 g of dehydrated media (BK010) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Divide into 16 x 200mm tubes, 10 mL broth per tube and add a Durham tube to each.

✓ **Reconstitution: 35,6 g/L**
✓ **Sterilization: 15 min at 121°C**

Double strength broth

- Dissolve 71,2 g of dehydrated media (BK010) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Divide into 20 x 200 mm tubes,10 mL broth per tube and add a Durham tube to each.
- Sterilize all tubes in an autoclave at 121°C for 15 min.
- Cool to room temperature.
- After cooling, the Durham tubes should not contain any trapped air.

✓ **Reconstitution: 71,2 g/L**
✓ **Sterilization: 15 min at 121°C**

Note:
For the enumeration of presumptive *Escherichia coli* in milk and dairy products, (NF ISO 11866-1), the broth should be supplemented with:
- 0.1 g/L of MUG (4-methyl-umbellifryl-ß-D glucuronide, supplement BS02408) and 1.0 g/L of tryptophan, at single strength.
- 0.2 g/L de MUG and 2,0 g/L of tryptophan, at double strength.

5 INSTRUCTIONS FOR USE

- Inoculate the tubes at double strength prepared as above or ready-to-use (BM098) with 10 mL of sample inoculum.
- Inoculate the tubes at single strength prepared as above or ready-to-use (BM097) with 1 mL of sample inoculum and its serial dilutions.
- Incubate for 24 ± 2 hours at 30 ± 1 °C or at 37 ± 1 °C, following the directive or the accords in vigor. If the tubes cannot be considered positive after 24 hours, extend the incubation to 48 hours.

✓ **Inoculation:**
- Double strength media: 10 mL
- Single strength media: 1 mL

✓ **Incubation:**
24 to 48 h at 30 or 37 °C

6 RESULTS

Lactose fermentation, shown by the presence of gas in the Durham tubes in less than 48 hours, indicates the presence of coliform bacteria. Verification may be carried out by isolating and identifying on an appropriate medium. Enumerate by using the most probable number method.

7 QUALITY CONTROL

Dehydrated media: off-white powder, free-flowing and homogeneous.
Prepared media: amber, limpid solution.

Typical culture response after 24 to 48 hours of incubation at 30 °C (NF EN ISO 11133; FD T 90-461):

Microorganisms		Growth	Gas production (Durham tube)
(1) *Escherichia coli*	WDCM 00012	Good, score 2	≥ 5 mm
(1) *Citrobacter freundii*	WDCM 00006	Good, score 2	≥ 5 mm
Enterococcus faecalis	WDCM 00087	Partially inhibited, score 0-1	Negative

(1) inoculum ≤10^2 microorganisms.

Typical culture response after 24-48 hours of incubation at 37°C

Microorganisms		Growth	Gas production (Durham tube)
(1) *Escherichia coli*	WDCM 00012	Good, score 2	≥ 5 mm
Enterococcus faecalis	WDCM 00087	Partially inhibited, score 0-1	Negative

(1) inoculum ≤10^2 microorganisms

8 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
Ready-to-use media (single and double strength): 15-25 °C.
The expiration dates are indicated on the labels.

Prepared media in tubes (*): 180 days at 15-25 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

Refrigeration of the tubes is not advised as they could trigger the formation of cloudiness and precipitates.

9 PACKAGING

Dehydrated media:
500 g bottle BK010HA

Ready-to-use media each containing a Durham tube.
50 x 10 mL tubes (simple concentration)..... BM09708
50 x 10 mL (double strength)..... BM09808

10 BIBLIOGRAPHY

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NF T90-413. Octobre 1985. Essais des eaux. Recherche et dénombrement des coliformes et des coliformes thermotolérants. Méthode générale par ensemencement en milieu liquide (NPP).

NF ISO 7251. Juillet 2005. Microbiologie des aliments. Méthode horizontale pour la recherche et le dénombrement d'*Escherichia coli* présumés. Technique du nombre le plus probable.

NF ISO 11866-1. Septembre 2006. Lait et produits laitiers. Dénombrement d'*Escherichia coli* présumés. Partie 1 : Technique du nombre le plus probable avec utilisation de 4-méthylumbelliféryl-β-D-glucuronide (MUG).

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11 ADDITIONAL INFORMATION

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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