

TECHNICAL DATA SHEET

GLUTAMATE BROTH

ENUMERATION OF β -GLUCURONIDASE POSITIVE *ESCHERICHIA COLI*

1 INTENDED USE

Glutamate broth is used enumeration of β -glucuronidase positive *E. coli* in food products by the MPN technique. It is recommended when the microorganisms are found in a sub-optimal state due to exposition to stress including freezing, desiccation, disinfection or high salinity.

The described method does not allow the detection of strains of *Escherichia coli* that do not develop at 44°C, and, in particular, those that are β -glucuronidase negative, as the case of *E. coli* O157 and a few other strains of pathogenic *E. coli*.

The typical composition corresponds to that described in the standard NF EN ISO 16649-3.

2 HISTORY

In 1948, Folpmers was the first to develop a chemically defined medium based on the use of glutamic acid for the enumeration of coliform bacteria in water. The medium was subsequently modified by Gray and found to be better than MacConkey broth for the detection of *Escherichia coli* in chlorinated and non-chlorinated water.

3 PRINCIPLES

The presence of coliform bacteria is shown by gas production from lactose, as well as the color change of bromcresol purple to yellow.

Enterococci are inhibited by the absence of nutrients essential for their growth.

4 TYPICAL COMPOSITION

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

For 1 liter of media:

| | |
|--------------------------------------|---------|
| - Sodium glutamate | 6,35 g |
| - Ammonium chloride | 2,50 g |
| - Sodium formate | 0,25 g |
| - L(-) cystine..... | 20,0 mg |
| - L(-) aspartique acid | 24,0 mg |
| - L(+) arginine (hydrochloride)..... | 20,0 mg |
| - Lactose..... | 10,0 g |
| - Thiamine..... | 1,00 mg |
| - Nicotinic acid | 1,00 mg |
| - Pantothenic acid..... | 1,00 mg |
| - Magnesium sulfate | 0,10 g |
| - Ferric ammonium citrate | 10,0 mg |
| - Calcium chloride | 10,0 mg |
| - Dipotassium phosphate | 0,90 g |
| - Bromocresol purple | 10,0 mg |

pH of the ready-to-use media at 25 °C: 6.7± 0.1.

5 PREPARATION

Single strength broth

- Suspend 20,2 g of dehydrated media (BK186) in 1 liter of distilled or demineralized water for single strength broth.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes 16 x 160 mm, 10 mL per tube.

✓ **Reconstitution:**
20,2 g/L

Double strength broth

- Suspend 40,4 g of dehydrated media (BK186) in 1 liter of distilled or demineralized water, for double strength broth.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes 20 x 200 mm, 10 mL per tube.

✓ **Reconstitution:**
40,4 g/L

- Sterilize all the tubes in an autoclave at 116 °C for 10 min.
- Cool to room temperature.

✓ **Sterilization:**
10 min at 116 °C

Note: The sterilization can be raised to 15 minutes at 115 °C.

6 INSTRUCTIONS FOR USE

- Inoculate a tube of broth (for the detection) and 3 to 5 tubes (for enumeration) of double strength prepared with 10 mL of inoculum.
- Inoculate 3 to 5 tubes of single strength broth prepared with 1 mL of inoculum and its serial dilutions.
- Incubate for 24 ± 2 hours at 37 ± 1 °C.

✓ **Inoculation:**
- double strength: 10 mL
- single strength: 1 mL

✓ **Incubation:**
24 h at 37 °C

7 RESULTS

Tubes demonstrating a color change from the control tube or an acidification (tubes turning yellow) are considered as presumptive positive for *Escherichia coli*.

Re-inoculate each presumptive tube onto TBX agar (BK146) previously poured into Petri plates.

Incubate 22 ± 2 h at 44 ± 1 °C.

Characteristic colonies are blue.

8 QUALITY CONTROL

Dehydrated media: bluish-gray powder, free-flowing to slightly clumped, homogeneous.

Prepared media: blue-violet solution, limpid.

Typical culture response after 24 hours of incubation at 37°C (NF EN ISO 11133):

| Microorganisms | Growth |
|------------------------------|-----------------------------------|
| <i>Escherichia coli</i> | Good, score 2 (tube turns yellow) |
| <i>Escherichia coli</i> | Good, score 2 (tube turns yellow) |
| <i>Enterococcus faecalis</i> | Inhibited, score 0 |

9 STORAGE / SHELF LIFE

Dehydrated media: 2-20 °C.

Tightly close the bottle after using in order to avoid any moisture entering into the powder.
The expiration date is indicated on the label.

Prepared media in tubes (*): 90 days at 2-8 °C, shielded from light.

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

10 PACKAGING

Dehydrated media:

500 g bottle BK186HA

11 BIBLIOGRAPHY

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NF EN ISO 16649-3. Juillet 2015. Microbiologie des aliments. Méthode horizontale pour le dénombrement des *Escherichia coli* β-glucuronidase-positives. Partie 3 : Technique du nombre le plus probable utilisant le bromo-5-chloro-4-indolyl-3-β-D-glucuronate.

12 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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