

# TECHNICAL DATA SHEET

## EE BROTH ACC. TO MOSSEL

### SELECTIVE ENRICHMENT FOR *ENTEROBACTERIACEAE*

#### 1 INTENDED USE

EE Broth, buffered and containing bile salts, brilliant green and glucose, proposed by Mossel, is a selective enrichment media for the enumeration by Most Probable Number of *Enterobacteriaceae* in food, animal and environmental products.

It is also recommended in the various harmonized Pharmacopeia for the detection of Gram negative bacteria that are resistant to bile salts.

The typical composition is that found in the European Pharmacopeia.

#### 2 HISTORY

At its origin, the formulation was developed by Mossel, Visser and Cornelissen in 1963. Glucose favors the growth of enterobacteria and notably that of *Shigella* and *Salmonella*, both lactose negative and positive strains. The enrichment technique in liquid media, which detects bacterial growth rather than gas production, allows the inclusion of those enterobacteria that do not systematically produce gas in other selective liquid media.

#### 3 PRINCIPLES

The simultaneous presence of bile salts and brilliant green leads to the inhibition of nearly all Gram positive bacteria as well as Gram negative strains other than enterobacteria.

Pancreatic digest of gelatin and glucose constitute the Nitrogen and energy sources, respectively, for the development of *Enterobacteriaceae*.

Phosphates allow a suitable buffering of the media in order to increase its capacity for recuperation.

#### 4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Pancreatic digest of gelatin .....	10,00 g
- Bacteriological beef bile .....	20,00 g
- Glucose .....	5,00 g
- Disodium phosphate anhydrous (*).....	6,45 g
- Monopotassium phosphate .....	2,00 g
- Brilliant green .....	15,0 mg

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

(\*) : 6.45 g of anhydrous disodium phosphate corresponds to 8 g of dihydrated disodium phosphate.

#### 5 PREPARATION

- Dissolve 43,5 g of dehydrated media (BK127) in 1 liter of distilled or demineralized water. .
- Stir slowly until complete dissolution.
- Divide into tubes, at 10 mL per tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**  
43,5 g/L

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

## 6 INSTRUCTIONS FOR USE

### Detection or enumeration of *Enterobacteriaceae* (NF ISO 21528-1)

- Transfer 1 mL of the enrichment broths to each tube of EE broth prepared as described.
- Incubate for 24 ± 2 hours at 37 °C.

✓ **Inoculation** : MPN  
1 mL / tube

✓ **Incubation** :  
24 h at 37°C

### Detection of Gram negative bacteria resistant to bile salts (Pharmacopie)

- Inoculate 1 mL of the primary dilution in 10 mL of EE broth.
- Incubate at 30-35 °C for 24-48 hours.

✓ **Inoculation** :  
1 mL / tube

✓ **Incubation** :  
24-48 h at 30-35°C

## 7 RESULTS

Observe the tubes for bacterial growth.

Restreak the tubes onto VRBG agar (BK011, BM075), using a loop of culture. If the plates present characteristic colonies after suitable incubation, proceed with the recommended confirmation tests.

## 8 QUALITY CONTROL

**Dehydrated media** : beige to greenish powder, free-flowing and homogeneous.

**Prepared media** : limpid, green solution.

Typical culture response after 24 hours incubation at 37 °C, followed by subculturing (NF EN ISO 11133) :

Microorganisms		Growth
<i>Escherichia coli</i> + <i>Enterococcus faecalis</i>	WDCM 00012 WDCM 00087	> 10 characteristic colonies on VRBG
<i>Salmonella</i> Typhimurium + <i>Enterococcus faecalis</i>	WDCM 00031 WDCM 00087	> 10 characteristic colonies on VRBG
<i>Enterococcus faecalis</i>	WDCM 00087	Total inhibition on TSA

Typical culture response after 24 hours of incubation at 30-35 °C, inoculum < 100 microorganisms :

Microorganisms		Growth
<i>Escherichia coli</i>	WDCM 00012	Positive
<i>Pseudomonas aeruginosa</i>	WDCM 00026	Positive
<i>Staphylococcus aureus</i>	WDCM 00032	Inhibited

## 9 STORAGE / SHELF LIFE

**Dehydrated media** : 2-30 °C.

The expiration date is indicated on the package tongue.

**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 ..... BK127HA

## 11 BIBLIOGRAPHY

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Mossel, D.A.A., Visser, M., and Cornelissen, A.M.R. 1963. The Examination of Foods for *Enterobacteriaceae* using a Test of the type Generally Adopted for the Detection of *Salmonellae*. Jour. of Appl. Bact., 24: 444-452.

NF ISO 21528-1. Décembre 2004. Microbiologie des aliments. Méthodes horizontales pour la recherche et le dénombrement des *Enterobacteriaceae*. Partie 1 : Recherche et dénombrement à l'aide de la technique NPP avec préenrichissement.

Pharmacopée Européenne. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.

## 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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