

## Technical Data Sheet

# Mossel Broth (EE-Broth)

Ordering number: 1.46462.0006

Mossel Broth is designed for selective enrichment of bile-tolerant, gram-negative bacteria and particularly Enterobacteriaceae from non-sterile pharmaceutical products, foodstuff and other materials.

Mossel Broth (EE-Broth) is available in bottles or tubes with different filling volumes and locking mechanisms:

- Mossel Broth (article number 146219): 17 ml-**tube**, filling volume 10 ml
- Mossel Broth (EE-Broth) (article number 146346): 17 ml-**tube**, filling volume 10 ml
- Mossel Broth (EE-Broth) (article number 146373): 125 ml-bottle with **screw cap**, filling volume 100 ml
- Mossel Broth (article number 146463): 125 ml-bottle with **screw cap**, filling volume 100 ml
- Mossel Broth (EE-Broth) (article number 146462): 250 ml-bottle with **screw cap**, filling volume 100 ml
- Mossel Broth (EE-Broth) (article number 146434): 1000 ml-bottle with **screw cap**, filling volume 1000 ml

## Mode of Action

The undesired accompanying bacterial flora is almost completely inhibited by brilliant green and ox bile. Glucose favours the growth of all Enterobacteriaceae and the high buffering capacity of the culture medium prevents the formed acid from killing the culture. Glucose as the main energy source supports generally the rapid growth of all Enterobacteriaceae whilst enzymatic digest of animal tissue provides nitrogen, vitamins and amino acids.

## Typical Composition

|                                                       |         |
|-------------------------------------------------------|---------|
| Pancreatic Digest of Gelatin                          | 10 g/l  |
| Dehydrated Ox Bile                                    | 20 g/l  |
| Na <sub>2</sub> HPO <sub>4</sub> · 2 H <sub>2</sub> O | 8 g/l   |
| KH <sub>2</sub> PO <sub>4</sub>                       | 2 g/l   |
| Glucose Monohydrate                                   | 5 g/l   |
| Brilliant green                                       | 15 mg/l |
| pH at 25°C                                            | 7-7.4   |

The appearance of the medium is clear and bottle-green.

The medium can be adjusted and/or supplemented according to the performance criteria required.

## Application and Interpretation

### Pharmacopoeia application

According to the current, harmonized chapters of EP, USP and JP the presence of bile-tolerant, gram-negative bacteria is demonstrated by the preparation of a one to ten solution in Tryptic Soy Broth (e.g. article number 146334) of the sample to be tested.

For resuscitation the homogenate is incubated at 20-25 °C for 2 to a maximum of 5 hours. 10 ml out of this homogenate are inoculated into Mossel Broth. The medium is incubated at 30-35 °C for 18-48 hours.

For quantitative evaluation according to the harmonized Pharmacopoeia the tubes with a filling volume of 10 ml are inoculated with different dilutions steps of the homogenate in order to determine the MPN of bile-tolerant, gram-negative bacteria. The medium is incubated at 30-35 °C for 18-48 hours.

For direct enrichment of *E. coli* it is also possible to incubate the medium at 44° C in addition to a sample which is incubated at 30 to 37 °C.

An aliquot is subcultured on Violet Red Bile Glucose (VRBG) Agar - LI (article number 146000) and incubated at 30-35 °C for 18-24 hours.

The product complies, if there is no growth of colonies. For quantitative tests the smallest quantity of product which gives a positive result and the largest quantity which gives a negative result is noted and the probable number of bacteria is determined.

### Food application

Depend on the purpose for which the medium is used.

Allow the Mossel (EE) broth to equilibrate at room temperature if it was stored at a lower temperature.

Acc. to EN ISO 21528-1, transfer 1 ml of the culture obtained in the pre-enrichment (Buffered Peptone Water) to a tube containing 10 ml of EE broth. Minimize the transfer of particulate material from the pre-enrichment into the selective enrichment medium.

Incubate the inoculated broth under aerobic conditions, e.g. acc. to EN ISO 21528-1 at  $37 \pm 1$  °C for 24 h  $\pm$  2 h.

From the culture obtained in EE broth Violet Red Bile Glucose (VRBG) agar is inoculated for confirmation, see details given by EN ISO 21528-1.

### Storage and Shelf Life

The product can be used for tests until the expiry date if protected from light and properly sealed at +2 °C to +25 °C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

### Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).



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## Quality Control

| Control Strains               | ATCC # | Inoculum CFU | Incubation          | Expected Results                  |
|-------------------------------|--------|--------------|---------------------|-----------------------------------|
| <i>Escherichia coli</i>       | 8739   | 10-100       | 20-24 h at 33-35 °C | good growth; pronounced turbidity |
| <i>Pseudomonas aeruginosa</i> | 9027   | 10-100       | 20-24 h at 33-35 °C | good growth; pronounced turbidity |
| <i>Staphylococcus aureus</i>  | 6538   | 1000-10,000  | 44-48 h at 33-35 °C | no growth; no turbidity           |
| <i>Salmonella Typhimurium</i> | 14028  | 10-100       | 20-24 h at 35-37 °C | good growth; pronounced turbidity |
| <i>Enterococcus faecalis</i>  | 19433  | 1000-10,000  | 20-24 h at 35-37 °C | no growth; no turbidity           |

Please refer to the actual batch related Certificate of Analysis.

## Literature

European Directorate for the Quality of Medicines and Healthcare. (2014): The European Pharmacopoeia. 8th Ed. Chapter 2.6.12 Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Japanese Ministry of Health, Labour and Welfare. (2011): The Japanese Pharmacopoeia. 16th Ed. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count and II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

ISO/TS 11133-2 (2004-10): Microbiology of food and animal feeding stuffs - Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.

ISO 21528-1 (2004-08): Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 1: Detection and enumeration by MPN technique with pre-enrichment.

Mossel D.A.A., Vissar M. and Cornellisen A.M.R. (1963). The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of *Salmonellae*. J. Appl. Bacteriol. **26**: 444-452.

**Manafi, M.** 2012. Culture media for detection and Enumeration of “Total” *Enterobacteriaceae*, *Coliforms*, and *Escherichia coli* from Foods. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds)., pp. 233 - 260. Royal Society of Chemistry, Cambridge, UK.

United States Pharmacopoeial Convention. (2014): The United States Pharmacopoeia 38/National Formulation 33, Supp. 2. Chapter <61> Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter <62> Microbiological examination of non-sterile products: Test for specified products. Rockville, Md., USA.



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## Ordering Information

| Product                                                              | Cat. No.     | Pack size          |
|----------------------------------------------------------------------|--------------|--------------------|
| Mossel Broth (EE-Broth)                                              | 1.46462.0006 | 6 x 100 ml bottle  |
| Mossel Broth (EE-Broth)                                              | 1.46346.0020 | 20 x 10 ml tube    |
| Mossel Broth                                                         | 1.46219.0020 | 20 x 10 ml tube    |
| Mossel Broth                                                         | 1.46219.0100 | 100 x 10 ml tube   |
| Mossel Broth                                                         | 1.46463.0010 | 10 x 100 ml bottle |
| Mossel Broth (EE-Broth)                                              | 1.46373.0010 | 10 x 100 ml bottle |
| Mossel Broth (EE-Broth)                                              | 1.46434.0006 | 6 x 1000 ml bottle |
| Tryptic Soy Broth                                                    | 1.46334.0006 | 6 x 90 ml bottle   |
| Violet Red Bile Glucose (VRBG) Agar - LI                             | 1.46000.0020 | 20 x 90 mm plates  |
| Violet Red Bile Glucose (VRBG) Agar - LI                             | 1.46000.0120 | 120 x 90 mm plates |
| <b>ReadyPlate™</b> Violet Red Bile Glucose (VRBG) Agar acc ISO 21528 | 1.46127.0020 | 20 x 90 mm plates  |
| <b>ReadyPlate™</b> Violet Red Bile Glucose (VRBG) Agar acc ISO 21528 | 1.46127.0100 | 100 x 90 mm plates |

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