

Technical Data Sheet

GranuCult™

EE (Enterobacteriaceae Enrichment) MOSSEL Broth

acc. ISO

Ordering number: 1.05394.0500 / 1.05394.5000

For the selective enrichment of *Enterobacteriaceae* from food and animal feed and other materials.

This culture medium complies with the specifications given by EN ISO 21528-1.

Mode of Action

The undesired accompanying bacterial flora is almost completely inhibited by brilliant green and ox bile. Glucose favors 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

| Specified by ISO 21528 | | GranuCult™ EE (Enterobacteriaceae Enrichment) MOSSEL Broth acc. ISO | |
|------------------------------------|------------|---|------------|
| Enzymatic Digest of Animal Tissues | 10 g/l | Enzymatic Digest of Animal Tissues | 10 g/l |
| Glucose | 5 g/l | D(+)-Glucose | 5 g/l |
| Beef Bile for Bacteriological Use | 20 g/l | Ox Bile* | 20 g/l |
| Brilliant Green | 0.0135 g/l | Brilliant Green | 0.0135 g/l |
| Na ₂ HPO ₄ | 6.45 g/l | Na ₂ HPO ₄ x 2 H ₂ O | 8.0 g/l |
| KH ₂ PO ₄ | 2 g/l | KH ₂ PO ₄ | 2 g/l |
| Water | 1000 ml/l | Water | n/a |
| pH at 25 °C | 7.2 ± 0.2 | pH at 25 °C | 7.2 ± 0.2 |

* Ox bile is equivalent to other different terms of bile.



Preparation

Dissolve 45 g in 1 l of purified water, fill into tubes, autoclave gently for 5 min at 121 °C or heat in a water bath at 100 °C for 30 min. Cool immediately.

Dispense the medium in 10 ml amounts into sterile tubes of appropriate capacity.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

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

According 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 36-38 °C for 22-26 h.

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

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According to EN ISO 21528, self-prepared medium in screw-capped containers can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to one month.

Quality Control

| Function | Control strains | Incubation | Method of control | Criteria | Expected results |
|--------------|---|-----------------------------------|-------------------|---|---|
| Productivity | <i>Escherichia coli</i> ATCC® 25922 + <i>Enterococcus faecalis</i> ATCC® 29212 | 22-26 h at 36-38 °C aerobic | Qualitative | >10 colonies on Violet Red Bile Glucose Agar | Pink to red colonies with or without precipitation halo on VRBD (Violet Red Bile Dextrose) agar acc. ISO 21528 |
| | <i>Escherichia coli</i> ATCC® 8739 + <i>Enterococcus faecalis</i> ATCC® 19433 | | | | |
| | <i>Salmonella</i> Typhimurium ATCC® 14028 + <i>Enterococcus faecalis</i> ATCC® 29212 | | | | |
| | <i>Salmonella Enteritidis</i> ATCC® 13076 + <i>Enterococcus faecalis</i> ATCC® 19433 | | | | |

| Function | Control strains | Incubation | Method of control | Criteria | Expected results |
|-------------|---|-----------------------------------|-------------------|--|------------------|
| Selectivity | <i>Enterococcus faecalis</i> ATCC® 19433 | 22-26 h at 36-38 °C aerobic | | Total inhibition on Tryptic Soy Agar (TSA) | - |
| | <i>Enterococcus faecalis</i> ATCC® 29212 | | | | |

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133.

Literature

ISO International Standardisation Organisation. 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. EN ISO 21528-1:2004.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

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.

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 adapted for the detection of salmonellae. J. Appl. Bact. **24**: 444-452.

Mossel, D.A.A., Harrewijn, G.A. and Nesselroy-van Zadelhoff, C.F.M. (1974): Standardisation of the selective inhibitory effect of surface active compounds used in media for the detection of *Enterobacteriaceae* in food and water. Health Lab. **11**: 260-267.

Ordering Information

| Product | Cat. No. | Pack size |
|--|--------------|-----------|
| GranuCult™ EE (Enterobacteriaceae Enrichment) MOSSEL Broth acc. ISO | 1.05394.0500 | 500 g |
| GranuCult™ EE (Enterobacteriaceae Enrichment) MOSSEL Broth acc. ISO | 1.05394.5000 | 5 kg |
| Granucult™ Nutrient Agar acc. ISO 6579, ISO 10273 and ISO 21528 | 1.05450.0500 | 500 g |
| GranuCult™ Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP | 1.07228.0500 | 500 g |
| GranuCult™ VRBD (Violet Red Bile Dextrose) Agar acc. EP, USP, JP and ISO 21528 | 1.10275.0500 | 500 g |

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