

Technical Data Sheet

GranuCult™

VRB (Violet Red Bile Lactose) Agar

acc. ISO 4832 and FDA-BAM

Ordering number: 1.01406.0500 / 1.01406.5000

For the detection and colony counting of coliform bacteria from food and animal feed, water and other materials.

This culture medium complies with the specifications given by ISO 4832, FDA-BAM and APHA.

Violet Red Bile (VRB) agar is also called Violet Red Bile Lactose (VRBL) agar or Crystal Violet Neutral red Bile Lactose agar.

Mode of Action

Crystal violet and bile salts inhibit growth primarily of the Gram-positive accompanying bacterial flora. Degradation of lactose to acid is indicated by the pH indicator neutral red which changes its color to red and by precipitation of bile acids. Enzymatic digest of animal tissue provides carbon and nitrogen sources for the growth and yeast extract primarily supplies the B-complex vitamins whilst agar is the solidifying agent.

Typical Composition

| Specified by ISO 4832 | | FDA-BAM M174 | | GranuCult™ VRB Agar acc. ISO 4832 and FDA-BAM | |
|------------------------------------|-----------|--------------------------------|-----------|--|-----------|
| Enzymatic Digest of Animal Tissues | 7 g/l | Peptone or Gelysate | 7 g/l | Enzymatic Digest of Animal Tissues | 7 g/l |
| Yeast Extract | 3 g/l | Yeast Extract | 3 g/l | Yeast Extract | 3 g/l |
| Bile Salts | 1.5 g/l | Bile Salts or Bile Salts No. 3 | 1.5 g/l | Bile Salts | 1.5 g/l |
| NaCl | 5 g/l | NaCl | 5 g/l | NaCl | 5 g/l |
| Lactose | 10 g/l | Lactose | 10 g/l | Lactose | 10 g/l |
| Neutral Red | 0.03 g/l | Neutral Red | 0.03 g/l | Neutral Red | 0.03 g/l |
| Crystal Violet | 0.002 g/l | Crystal Violet | 0.002 g/l | Crystal Violet | 0.002 g/l |
| Agar | 12-18 g/l | Agar | 15 g/l | Agar-Agar* | 13 g/l |
| Water | 1000 ml/l | Water | 1000 ml/l | Water | n/a |
| pH at 25 °C | 7.2 ± 0.2 | pH at 25 °C | 7.2 ± 0.2 | pH at 25 °C | 7.2 ± 0.2 |

* Agar-agar is equivalent to other different terms of agar.

Preparation

Dissolve 39.5 g in 1 l of purified water. Heat in boiling water and agitate frequently until completely dissolved. Afterwards do not boil for more than 2 minutes.

Do not autoclave. Do not overheat!

If the medium is to be used immediately for poured plate technique, cool it to 44-47 °C in a water bath before use. Use the molten medium as soon as possible, it should not be retained for more than 4 h, as specified by ISO 4832 and EN ISO 11133.

If the medium is used for surface plating technique, there should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

The prepared medium is clear and red. The pH value at 25 °C is in the range of 7.0-7.4.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used. VRB agar is usually inoculated by poured plate techniques, e.g. as described by ISO 4832.

Incubate the inoculated plates under aerobic conditions. e.g. acc. to EN ISO 4832 at 29-31 °C or at 36-38 °C for 22-26 h.

Coliform bacteria will produce purplish-red colonies of at least 0.5 mm diameter, sometimes surrounded by a reddish zone of precipitated bile. These are considered as typical colonies of coliforms and do not require further confirmation.

The appearance of a reddish zone of precipitated bile around the colonies depends on the type of coliforms.

Atypical colonies (e.g. smaller size than 0.5 mm) should be counted and confirmed, e.g. following the procedure as described by ISO 4832 by using BRILA broth (article number 1.05454.0500).

When testing products containing sugars other than lactose, presence of these sugars may lead to false positive results, in which case colonies should be confirmed.

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 Corry et al. (2012), self-prepared plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to 5 days.



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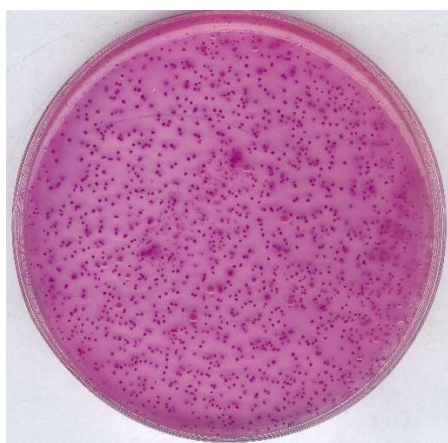
Quality Control

| Function | Control strains | Incubation | Reference medium | Method of control | Expected results |
|--------------|--|-----------------------------------|---------------------------|-------------------|---|
| Productivity | <i>Escherichia coli</i> ATCC® 8739 | 22-26 h at 29-31 °C aerobic | Tryptic Soy Agar (TSA) | Quantitative | Recovery ≥ 50 %, purplish-red colonies with or without precipitation halo |
| | <i>Escherichia coli</i> ATCC® 25922 | | | | |
| | <i>Enterobacter cloacae</i> ATCC® 13047 | | | | |
| Selectivity | <i>Enterococcus faecalis</i> ATCC® 19433 | 22-26 h at 29-31 °C aerobic | - | Qualitative | Total inhibition |
| | <i>Enterococcus faecalis</i> ATCC® 29212 | | | | |
| Specificity | <i>Pseudomonas aeruginosa</i> ATCC® 27853 | 22-26 h at 29-31 °C aerobic | - | Qualitative | No limit for recovery specified, colorless to beige colonies |

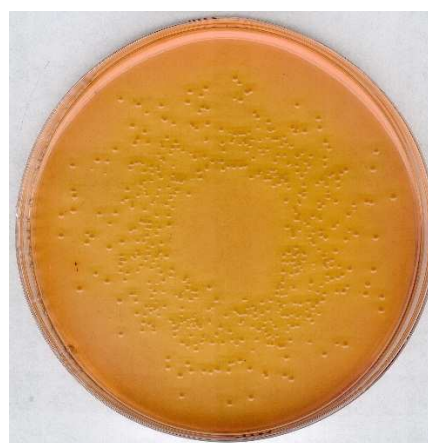
Please refer to the actual batch related Certificate of Analysis.

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

A recovery rate of 50 % is equivalent to a productivity value of 0.5.



Escherichia coli
ATCC® 11775



Salmonella typhimurium
ATCC® 14028



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Literature

APHA (2015) Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

APHA (2004) Standard Methods for the Examination of Dairy Products. 17th ed. American Public Health Association, Washington, D.C.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Handbook of Culture Media for Food and Water Microbiology, pp. 959-961. Royal Society of Chemistry, Cambridge, UK.

FDA-BAM (2002): Chapter No. 4: Enumeration of Escherichia coli and the Coliform Bacteria. U.S. Food and Drug Administration – Bacteriological Analytical Manual.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms – Colony-count technique. ISO 4832:2006.

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

Ordering Information

| Product | Cat. No. | Pack size |
|---|--------------|--------------------|
| GranuCult™ VRB (Violet Red Bile Lactose) Agar acc. ISO 4832 and FDA-BAM | 1.01406.0500 | 500 g |
| GranuCult™ VRB (Violet Red Bile Lactose) Agar acc. ISO 4832 and FDA-BAM | 1.01406.5000 | 5 kg |
| ReadyTube™ 200 VRBL Agar | 1.46423.0006 | 6 x 200 ml bottles |
| GranuCult™ BRILA (Brilliant-Green Bile Lactose) Broth acc. ISO 4831, ISO 4832 and FDA-BAM | 1.05454.0500 | 500 g |
| GranuCult™ BRILA (Brilliant-Green Bile Lactose) Broth acc. ISO 4831, ISO 4832 and FDA-BAM | 1.05454.5000 | 5 kg |

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