

## Technical Data Sheet

### ReadyTube™ 200 VRBL (Violet Red Bile Lactose) Agar acc. ISO 4832 and FDA-BAM

Ordering number: 1.46423.0006

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 21528		FDA-BAM M174		ReadyTube™ 200 VRBL	
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 No. 3	1.5 g/l	Bile salts or Bile Salts No. 3	1.5 g/l	Bile Salts No. 3	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	14 g/l
Water	1000 ml/l	Water	1000 ml/l	Water	1000 ml/l
pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.2 ± 0.2	pH at 25 °C	7.2 ± 0.2

## Application and Interpretation

The medium can be melted by placing in a boiling water bath as specified in ISO 11133. *Note: Avoid over heating the medium. Remove it from the boiling water bath once melted.* Transfer the molten medium in a thermostatically controlled water bath. Maintain temperature from 47°C to 50°C. It is recommended to use the medium as soon as possible.

*Preparation:* Heat the bottled agar in steam or boiling water bath approx. 45 min. **Caution:** avoid excessive or prolonged heating. Cool the molten agar down to 45-50 °C in a water bath and maintain temperature until use. Use the molten medium within 4 h of its preparation.

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 and Shelf Life

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

## 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.).

## 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	Tryptic Soy Agar (TSA)	Quantitative	Recovery $\geq 50$ %, purplish-red colonies with or without precipitation halo
	<i>Escherichia coli</i> ATCC 25922				
Selectivity	<i>Enterococcus faecalis</i> ATCC 19433	22-26 h at 29-31 °C	Tryptic Soy Agar (TSA)	Qualitative	Total inhibition
	<i>Enterococcus faecalis</i> ATCC 29212				
Specificity	<i>Pseudomonas aeruginosa</i> ATCC 27853	22-26 h at 29-31 °C	Tryptic Soy Agar (TSA)	Qualitative	No recovery limit 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.

## Literature

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

APHA (2004): Standard Methods for the Examination of Dairy Products. 17<sup>th</sup> 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 4832:2006:** Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms - Colony-count technique.

**ISO 11133:2014:** Microbiology of food and animal feed and water – Preparation, production, storage and performance testing of culture media

**ISO 7218 AMD 1: 2013:** Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

## Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
ReadyTube™ 200 VRBL Agar	1.46423.0006	6 x 200 ml	
GranuCult™ VRB (Violet Red Bile Lactose) Agar ISO 4832	1.01406.0500	500 g	5 kg
GranuCult™ BRILA (Brilliant Green Bile Lactose) Broth	1.05454.0500	500 g	5 kg

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