Technical Data Sheet

GranuCult™
VRBD (Violet Red Bile Dextrose) Agar
acc. EP, USP, JP and ISO 21528
Ordering number: 1.10275.0500

For the detection and enumeration of *Enterobacteriaceae* from food and animal feed and other materials and bile-tolerant Gram-negative bacteria from pharmaceutical and other materials.

This culture medium complies with the specifications given by EN ISO 21528 and APHA. It complies with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.

Violet Red Bile Dextose (VRBD) agar is also named as Violet Red Bile Glucose (VRBG) agar or Crystal Violet Neutral red Bile Glucose (Dextrose) agar.

Mode of Action

Crystal violet and bile salts inhibit the accompanying bacterial flora. Degradation of glucose is accompanied by production of acid, which is indicated by a color change to red and by zones of precipitated bile acids surrounding the colonies. All *Enterobacteriaceae* are detected as they all degrade glucose to acid. The culture medium is not, however, absolutely specific for these organisms as some other accompanying bacteria (e.g. *Aeromonas*) also show these reactions. 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 and APHA		Specified by EP, USP, JP		GranuCult™ VRBD (Violet Red Bile Dextrose) agar acc. EP, USP, JP and ISO 21528	
Enzymatic Digest of Animal Tissues	7 g/l	Pancreatic Digest of Gelatin	7 g/l	Pancreatic Digest of Gelatin (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	1.5 g/l	Bile Salts*	1.5 g/l
NaCl	5 g/l	NaCl	5 g/l	NaCl	5 g/l
Glucose	10 g/l	Glucose Monohydrate	10 g/l	D(+)-Glucose	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	9-18 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	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

* Bile salts include bile salts No. 3 (see EN ISO 11133).

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

Preparation

Dissolve 39.5 g in 1 liter 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 EN ISO 21528 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.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

VRBD agar is usually inoculated by poured plate techniques or by surface plating technique with or without precious enrichment step(s), e.g. as described by EN ISO 21528 and by EP, USP and JP.

Incubate the inoculated plates under aerobic conditions. e.g. acc. to EN ISO 21528 at 36-38 °C for 22-26 h or at 30-35 °C for 18-24 h according to EP, USP and JP.

Characteristic colonies are pink to red or purple with or without precipitation halos.

Certain *Enterobacteriaceae* may cause decoloration of their colonies or of the medium. Therefore, when no characteristic colonies are present, choose whitish colonies for confirmation.

For confirmation follow the methods given by the appropriate standard, e.g. by EN ISO 21528.

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 plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up to one month.

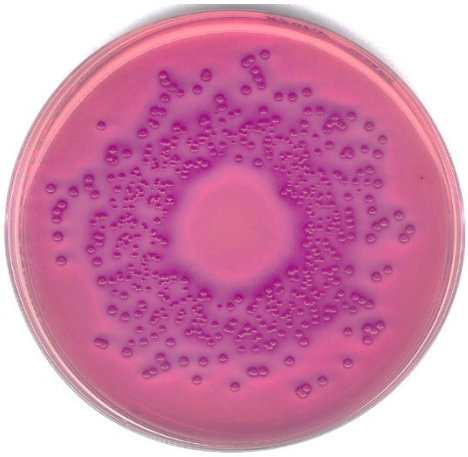
Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Escherichia coli* ATCC® 8739	22-26 h at 29-31 °C aerobic	Tryptic Soy Agar (TSA)	Quantitative	Recovery ≥ 50 %, pink to red colonies with or without precipitation halo
	Escherichia coli ATCC® 25922				
	Salmonella Typhimurium ATCC® 14028				
	Salmonella Enteritidis ATCC® 13076				
	Escherichia coli ATCC® 8739	up to 18 h at 30-35°C aerobic			Recovery ≥ 50 %, red colonies
	Pseudomonas aeruginosa ATCC® 9027				Recovery ≥ 50 %, nearly colorless to slightly red colonies
Selectivity	*Enterococcus faecalis* ATCC® 19433	22- 26 h at 29- 31 °C aerobic	-	Qualitative	Total inhibition
	Enterococcus faecalis ATCC® 29212				
	Staphylococcus aureus ATCC® 6538	at least 24 h at 30-35°C aerobic			No growth
	Bacillus cereus ATCC® 11778				

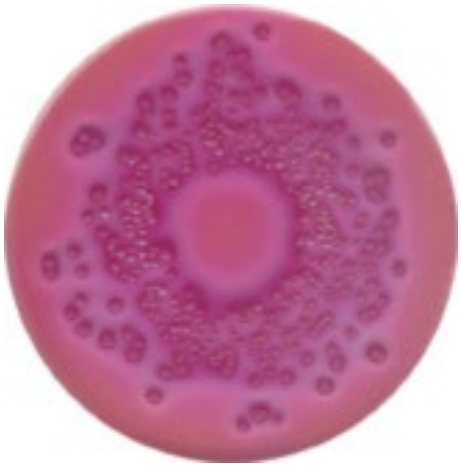
Please refer to the actual batch related Certificate of Analysis.

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.



Salmonella Typhimurium ATCC® 14028



Escherichia coli ATCC® 8739

Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th 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. 962-964. Royal Society of Chemistry, Cambridge, UK.

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

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 and animal feeding stuffs -- Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method. EN ISO 21528-2:2004.

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

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

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 Pharmacopeial Convention. (2014): The United States Pharmacopeia 38/National Formulation 33, Supp. 2. Chapter <62> Microbiological examination of non-sterile products: Test for specified products. Rockville, Md., USA.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult™ VRBD (Violet Red Bile Dextrose) Agar acc. EP, USP, JP and ISO 21528	1.10275.0500	500 g	
ReadyPlate™ VRBG Agar ISO 21528	1.46127.0020	20 x 90 mm	100 x 90 mm 200 ml
GranuCult™ Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	1.07228.0500	500 g	5 kg, 25 kg
ReadyTube™ 9 BPW ISO 6579, 6887, 21528	1.46142.0020	20 x 9 ml	100 x 9 ml, 6 x 225 ml, 6 x 1000 ml,
GranuCult™ Nutrient Agar acc. ISO 6579, ISO 10273 and ISO 21528	1.05450.0500	500 g	1 x 2000 ml
Standard I Nutrient Agar (in plates)	1.46256.0020	20 x 90 mm	
Bactident® Oxidase	1.13300.0001	50 strips	

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