Technical Data Sheet

GranuCult® prime

TCBS (Thiosulfate Citrate Bile Sucrose) agar acc. ISO 21872 and FDA-BAM

Ordering number: 1.03854.0500

For the isolation and differentiation of *Vibrio* spp. from products intended for human consumption and the feeding of animals and environmental samples in the area of primary production, food production and food handling and other materials.

TCBS (Thiosulfate Citrate Bile Sucrose) agar acc. ISO 21872 and FDA-BAM is also known as Vibrio selective agar.

This culture medium complies with the specifications given by EN ISO 21872-1, FDA-BAM Medium M147, GB 4789.7 and APHA.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

Mode of Action

This culture medium contains the selective components bile salts, thiosulfate, citrate and a relatively high pH of 8.6. The high concentrations of thiosulfate and citrate and the strong alkalinity of this medium largely inhibit the growth of *Enterobacteriaceae*. Ox bile and cholate suppress primarily enterococci.

Differentiation is based on sucrose fermentation, detected by the pH indicators, and H_2S production from thiosulfate. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H_2S-positive colonies to become black in color.
Vibrio species do not produce H_2S but, depending on the species, may ferment sucrose.
Any coliform bacteria, which may grow, cannot metabolize sucrose. Only a few sucrose-positive *Proteus* strains can grow to form yellow, vibrioid-like colonies. The mixed indicator thymol blue-bromothymol blue changes its color to yellow, when acid is formed, even in this strongly alkaline medium.
Peptone and yeast extract allow good growth and agar is the solidifying agent.

Typical Composition

APHA specifies no composition for TCBS (Thiosulfate Citrate Bile Sucrose) agar.

Specified by EN ISO 21872-1		Specified by FDA-BAM Medium M147, GB 4789.7		GranuCult® prime TCBS (Thiosulfate Citrate Bile Sucrose) agar acc. ISO 21872 and FDA-BAM	
Peptone	10.0 g/l	Peptone	10.0 g/l	Peptone	10.0 g/l
Yeast extract	5.0 g/l	Yeast extract	5.0 g/l	Yeast extract	5.0 g/l
Sodium citrate	10.0 g/l	Sodium citrate dihydrate	10.0 g/l	Sodium citrate dihydrate	10.0 g/l
Sodium thiosulfate	10.0 g/l	Sodium thiosulfate pentahydrate	10.0 g/l	Sodium thiosulfate pentahydrate	10.0 g/l
Dried bovine bile	8.0 g/l	Oxgall	5.0 g/l	Mixture from ox bile components* (containing Ox bile, dried 5.0 g/l and Sodium cholate 3.0 g/l)	8.0 g/l
		Sodium cholate	3.0 g/l		
Sucrose	20.0 g/l	Sucrose	20.0 g/l	Sucrose	20.0 g/l
Sodium chloride	10.0 g/l	Sodium chloride	10.0 g/l	Sodium chloride	10.0 g/l
Iron(III) citrate	1.0 g/l	Ferric citrate	1.0 g/l	Iron(III) citrate**	1.0 g/l
Thymol blue	0.04 g/l	Thymol blue	0.04 g/l	Thymol blue	0.04 g/l
Bromothymol blue	0.04 g/l	Bromothymol blue	0.04 g/l	Bromothymol blue	0.04 g/l
Agar-agar	8 g to 18 g/l ***	Agar	15.0 g/l	Agar-agar****	14.0 g/l
Water	1000ml	Water	1000ml	Water	n/a
pH at 25 °C	8.6 ± 0.2	pH at 25 °C	8.6 ± 0.2	pH at 25 °C	8.6 ± 0.2

* This mixture contains Ox bile and Sodium cholate to meet the required performance criteria, see EN ISO 11133:2014, clause 4.3.2.
** Iron(III)citrate is synonymous with Ferric citrate.
*** Depending on the gel strength of the agar-agar.
**** Agar-Agar is equivalent to other different terms of agar.
***** GB 4789.7 specifies the pH before autoclaving.

Preparation

Dissolve 88.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Do not autoclave!

The dehydrated medium is a granulate with green color.
The prepared medium is clear to slightly opalescent and blue-green.
The pH value at 25 °C is in the range of 8.4 – 8.8.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.
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.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 21872-1, FDA-BAM and APHA, inoculate the surface of the medium from the selective enriched cultures so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 21872-1 at (37 ± 1 °C) for (24 ± 3 h);
- acc. to FDA-BAM Chapter No. 9 at (35 ± 2 °C) for (18 – 24 h);
- acc. to GB 4789.7 at (36 ± 1 °C) for (18 – 24 h);
- acc. to APHA Chapter No. 40 at (35 ± 2 °C) overnight.

On TCBS (Thiosulfate Citrate Bile Sucrose) agar acc. ISO 21872 and FDA-BAM, *V. parahaemolyticus*, *V. vulnificus,* and *V. cholerae* exhibit different typical colony morphologies:

— typical colonies of *V. parahaemolyticus* and *V. vulnificus* are smooth, green (negative sucrose) and of 2 mm to 3 mm in diameter;
— typical colonies of *V. cholerae* are smooth, yellow (positive sucrose) and of 1 mm to 2 mm, often 2 mm to 3 mm in diameter.

Colonies of the most important bacteria usually have the appearance described below:

Appearance of colonies	**Microorganisms**
Flat, 2-3 mm in diameter, yellow	*Vibrio cholerae, Vibrio cholerae* Biotype El Tor
Small, blue-green centre	*Vibrio parahaemolyticus*
Large, yellow	*Vibrio alginolyticus*
Blue	*Pseudomonas, Aeromonas* and others
Very small, translucent	*Enterobacteriaceae* and others

This presumptive evidence must be confirmed by carrying out the usual tests.

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.

Self-prepared plates can be stored in the dark and protected against evaporation acc. to Corry et al. (2012) at (5 ± 3 °C) for up to 3 weeks.

Microbiological Performance

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

Test method: Performance testing of solid culture media - Qualitative testing

Test strain	Specification	
	Growth	Typical reaction
Vibrio parahaemolyticus ATCC® 17802 [WDCM 00037]	good	green colonies
Vibrio parahaemolyticus NCTC 10885 [WDCM 00185]	good	green colonies
Vibrio furnissii DSM 14383 [WDCM 00186]	good	yellow colonies
Escherichia coli ATCC® 25922 [WDCM 00013]	total inhibition	not applicable
Escherichia coli ATCC® 8739 [WDCM 00012]	total inhibition	not applicable
Escherichia coli ATCC® 11775 [WDCM 00090]	total inhibition	not applicable

Incubation: 24 ± 3 h at 37 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2015) Chapter No. 40: *Vibrio*. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. - Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*. EN ISO 21872-1:2017.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

FDA-BAM (2004): Chapter No. 9: *Vibrio*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

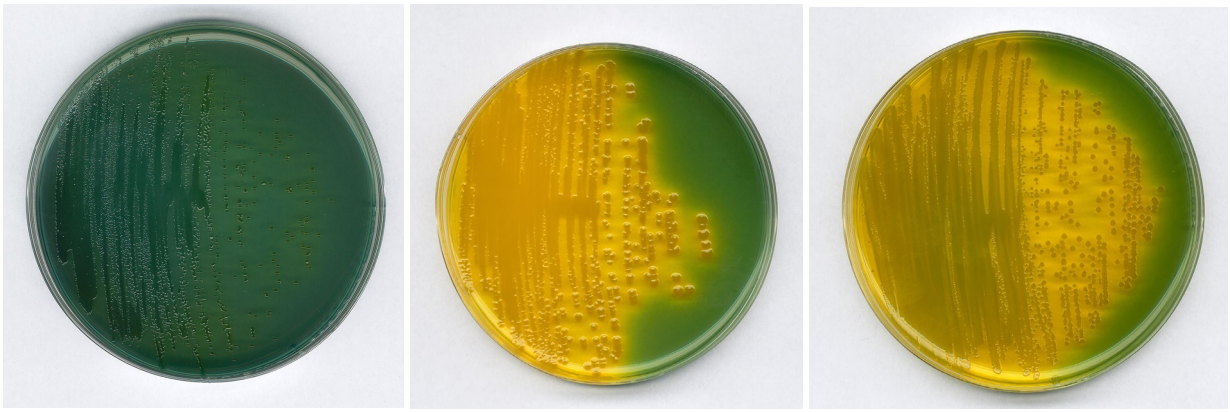
FDA-BAM (2018): Media Index for BAM - BAM Media M147: Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar. Food and Drug Administration - Bacteriological Analytical Manual.

National Health and Family Planning Commission of the People's Republic of China.
China Food and Drug Administration. National Standard of the People's Republic of China.
National food safety standard — Food microbiological examination: *Vibrio parahaemolyticus*.
GB 4789.7-2013.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Thiosulfate Citrate Bile salt Sucrose (TCBS) agar. In: Handbook of Culture Media for Food and Water Microbiology, pp. 942-944. Royal Society of Chemistry, Cambridge, UK.

Kobayashi, T., Enomoto, S., Sakazaki, R. and Kuwahara, S. (1963): A new selective isolation medium for pathogenic Vibrios: TCBS agar (modified Nakanashi's agar). Jpn. J. Bact. **18**: 387-391.

Oliver, J.D. (2012): Culture media for the isolation and enumeration of pathogenic *Vibrio* species in foods and environmental samples. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 377-402. Royal Society of Chemistry, Cambridge, UK.



Vibrio parahaemolyticus ATCC® 17802 [WDCM 00037]

Vibrio alginolyticus

Vibrio cholerae Biotype El Tor

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime TCBS (Thiosulfate Citrate Bile Sucrose) agar acc. ISO 21872 and FDA-BAM	1.03854.0500	500 g