Technical Data Sheet

GranuCult® prime
TSC (Tryptose Sulfite Cycloserine) Agar (base)
acc. ISO 15213, ISO 14189 and FDA-BAM

Ordering number: 1.11972.0500

For the enumeration and isolation of sulfite-reducing *Clostridium perfringens* by colony-count technique from samples belonging to the food chain, e.g. products intended for human consumption, products for feeding animals, environmental samples in the area of food and feed production and handling, samples from the primary production stage and by membrane filtration technique from samples of all types of water including water intended for human consumption.
TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM is also known as Sulfite cycloserin agar (SCA) and Tryptose sulfite agar as well as SFP (Shahidi Ferguson Perfringens) agar base.
This culture medium complies with the specifications given by EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024, EN ISO 14189:2013, ISO 6461-2:1986 resp. EN 26461-2:1993, EN ISO 7937:2004 (withdrawn), FDA-BAM Medium M169, AOAC Official Method 976.30:2023, GB 4789.13:2012 and APHA.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 peptone (for example, enzymatic digest of casein) providing carbon, nitrogen, vitamins and amino acids. Sodium disulfite (sodium metabisulfite) is reduced to sulfide by the enzyme sulfite reductase, produced by clostridia. The sulfide will then precipitate as a black deposit in the presence of iron(III) ammonium citrate. Agar is the solidifying agent.

TSC (Tryptose Sulfite Cycloserine) Agar utilises the selective inhibitory properties of D-Cycloserine which suppresses the most unwanted microorganisms.

SFP (Shahidi Ferguson Perfringens) agar contains polymyxin B and kanamycin as selective inhibitors of accompanying flora. Its selective inhibitory properties are slightly less than those of TSC (Tryptose Sulfite Cycloserine) Agar.

On TSC (Tryptose Sulfite Cycloserine) Agar and on SFP (Shahidi Ferguson Perfringens) agar, typical colonies of presumptive *Clostridium perfringens* appear black or grey to yellow-brown stained with sometimes only a faint colour. The presumptive colonies of *Clostridium perfringens* require confirmation according to the specified procedure.

TSC (Tryptose Sulfite Cycloserine) Agar can be used with the addition of Clostridium perfringens selective supplement (contains D-Cycloserine and MUP), Cat. No. 100888. The fluorogenic substrate MUP (4-Methylumbelliferylphosphate) enables the detection of alcaline and acid phosphatase. The phosphatase splits the fluorogenic substrate MUP and forms 4-methlyumbelliferone. This can be detected by its fluorescence in long wave UV light at 366 nm. The presence of acid phosphatase is a highly specific indicator for *Clostridium perfringens* and therefore a strong indication of the presence of *Clostridium perfringens*.

Sartory et al. (2006) reported that the results of their studies confirm that application of the acid phosphatase test is suitable for the confirmation of *C. perfringens* from water samples and are in agreement with the results of Eisgruber et al. (2000, 2003) for food analysis.

Typical Composition

Specified by EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024, EN ISO 14189:2013, EN ISO 7937:2004, GB 4789.13:2012.		**Specified by ISO 6461-2:1986, AOAC 976.30:2023, FDA-BAM Medium M169, APHA.**		**GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM**	
Peptone*	15 g/l	Tryptose*	15 g/l	Peptone including enzymatic digest of casein	15 g/l
Enzymatic digest of soya	5 g/l	Soytone	5 g/l	Enymatic digest of soya**	5 g/l
Yeast Extract	5 g/l	Yeast Extract	5 g/l	Yeast Extract	5 g/l
Sodium disulfite (sodium metabisulfite), anhydrous	1.0 g/l	Sodium disulfite (sodium metabisulfite), anhydrous	1.0 g/l	Sodium disulfite (sodium metabisulfite), anhydrous	1.0 g/l
Iron(III) ammonium citrate***	1.0 g/l	Iron(III) ammonium citrate***	1.0 g/l	Iron(III) ammonium citrate***	1.0 g/l
Agar	9.0-18 g/l****	Agar	9.0-18 g/l****	Agar-Agar*****	12 g/l
Water	1000 ml/l	Water	1000 ml/l	Water	n/a
Supplement to be added after autoclaving:					
D-Cycloserine	0.4 g/l	D-Cycloserine ******	0.4 g/l	D-Cycloserine	0.4 g/l
pH at 25 °C	7.6 ± 0.2	pH at 25 °C	7.6 ± 0.2	pH at 25 °C	7.6 ± 0.2

* EN ISO 15213-2 and EN ISO/TS 15213-3: For example, enzymatic digest of casein.
EN ISO 14189 specifies: Enzymatic digest of casein; GB 4789.13 specifies: Tryptone.
According EN ISO 11133, enzymatic digest of animal and plant tissue is equivalent to tryptose and enzymatic digest of casein is equivalent to tryptone.
EN ISO 7937:2004 (withdrawn) specifies enzymatic digest of protein.

**	Enzymatic digest of soy peptone is equivalent to soytone.
***	This reagent should contain at least 150 g/kg of iron.
****	Depending on the gel strength of the agar.
*****	Agar-Agar is equivalent to other different terms of agar.
******	ISO 6461-2:1986 specifies no addition of D-cycloserine.

Preparation

For the preparation of **TSC (Tryptose Sulfite Cycloserine) agar**, dissolve 39 g in 1 l of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave 15 minutes at 121 °C. At 44 °C to 47 °C, mix in 10 ml of a filter-sterilized 4% D-Cycloserine, Cat. No. C6880 or C30020, solution to obtain a final cycloserine concentration of 0.4 g per litre TSC agar.

For the preparation of **SFP (Shahidi Ferguson Perfringens) agar**, mix in 3 mg/l Polymyxin B sulfate, Cat. No. P1004 or P4932, and 12 mg/l Kanamycin sulfate, Cat. No. K1377, as filter-sterilized solutions to the sterilized TSC agar base at 44 °C to 47 °C.

For the preparation of **TSC agar with MUP**, mix in the dissolved content of 2 vials Clostridium perfringens selective supplement (contains D-cycloserine and MUP), Cat. No. 100888, to the sterilized TSC agar base at 44 °C to 47 °C.

For the preparation of **Tryptose sulfite agar acc. ISO 6461-2**, add no D-Cycloserine.

If the medium is to be used immediately for poured plate technique, cool it to 44 °C to 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 11133.

If the medium is to be used for surface plating or for membrane filtration technique, pour plates with a depth of at least 5 mm.

The dehydrated medium is a granulate with beige color.

The prepared medium is clear and brown. Due to the composition, precipitate may be visible in the prepared culture medium after sterilisation. This has no effect on the performance of the culture medium.

The pH value at 25 °C is in the range of 7.6 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 15213-2, use the pour plate technique with the medium cooled to 44 °C to 47 °C, with 12 ml to 15 ml for Petri dishes with a diameter of 90 mm or 30 ml to 35 ml for Petri dishes with a diameter of 140 mm. Allow to solidify by leaving the Petri dishes standing on a cool horizontal surface.

After complete solidification, pour about 5 ml of the medium for 90 mm Petri dishes or 12 ml for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium. Allow to solidify as specified above.

Incubate the inverted plates at (37 ± 1) °C in an anaerobic atmosphere for (20 ± 2) h.

Following the procedure given by EN ISO 14189, use the membrane filtration technique and place the membrane grid face upwards on a TSC agar plate ensuring that no air bubbles are trapped under the filter. Alternatively, a thin layer (about 5 ml to 10 ml related to a petri dish of 90 mm diameter) molten TSC agar (equilibrated in a water bath at (45 ± 1) °C) as an overlay on the filter can be used. Allow to solidify before anaerobic incubation. This procedure may enhance the blackening of the colonies. It is not necessary to add cycloserine in the TSC agar for the overlay. However, obtaining pure cultures for the confirmation test may be more laborious.

Incubate at (44 ± 1) °C in an anaerobic atmosphere for (21 ± 3) h.

NOTE: For every technique used, longer incubation can result in excess blackening of the plates.

Examine the plates for presumptive *Clostridium perfringens*: Typical colonies, which show black or grey to yellow-brown staining (even if the colour is faint) on or in the medium respective on or under the membrane filter, are counted.

Upon removal of the plates from the anaerobic atmosphere, plates shall be counted within 30 min as the colour of the colonies can rapidly fade and disappear upon exposure to oxygen. If anaerobic jars are used, the plates should be checked jar by jar or in small portions if the incubation was performed in an anaerobic incubator.

Diffuse, unspecific blackening of the medium can occur. The growth of anaerobic bacteria, which produce hydrogen (not H_2S), can also reduce the sulfite present and lead to a general blackening of the medium, which makes it difficult to count typical colonies.

For confirmation, follow the procedure, e.g. given by EN ISO 15213-2, EN ISO/TS 15213-3 or by EN ISO 14189.

According EN ISO 15213-2:2023 and EN ISO/TS 15213-3:2024, GranuCult® prime SIM (Sulfite Indole Motility) Agar acc. ISO 15213, Cat. No. 105470, can be used for confirmation.

Following the procedure for using TSC agar with MUP, use the pour plate technique with the medium cooled to 44 °C to 47 °C, with 12 ml to 15 ml for 90 mm Petri dishes or 30 ml to 35 ml for 140 mm Petri dishes. Allow to solidify by leaving the Petri dishes standing on a cool horizontal surface. Incubate at (44 ± 1) °C or at (37 ± 1) °C in an anaerobic atmosphere for (21 ± 3) h.

Fluorescence can be detected with an UV lamp at 366 nm: light blue fluorescing black or grey or yellow-brown stained colonies indicate *Clostridium perfringens* caused by the phosphate reaction with the methylumbelliferyl phosphate substrate.

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 15213-2:2023, EN ISO/TS 15213-3:2024 and EN ISO 14189, self-prepared base medium can be stored in closed containers or tubes at (5 ± 3) °C for up to four weeks in the dark. Prior to use, the stored medium is melted completely and cooled down before adding the supplement and mixed well before use.

According to EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024 and EN ISO 14189:2013, filter-sterilized D-cycloserine solution can be dispensed in suitable volumes and stored in closed containers at -20 °C for up to four weeks. Alternatively, the dispensed volumes can be stored at (-70 ± 10) °C for up to 12 months.

According to EN ISO 14189:2013, self-prepared TSC agar plates should be used as fresh as possible on the same day. If storage of the prepared plates is inevitable, the plates can be stored under anaerobic conditions at (5 ± 3) °C for up to seven days. Plates should be discarded, once removed from the refrigerator, if not used. Plates should be not returned to storage, as the performance of the medium deteriorates. Plates need to be dried well before use, following EN ISO 11133.

According to EN ISO/TS 15213-3:2024, self-prepared TSC agar plates can be stored at (5 ± 3) °C for up to four weeks in the dark.

Microbiological Performance

The performance test is in accordance with the current versions of EN ISO 11133, EN ISO 15213-2, EN ISO/TS 15213-3 and EN ISO 14189.

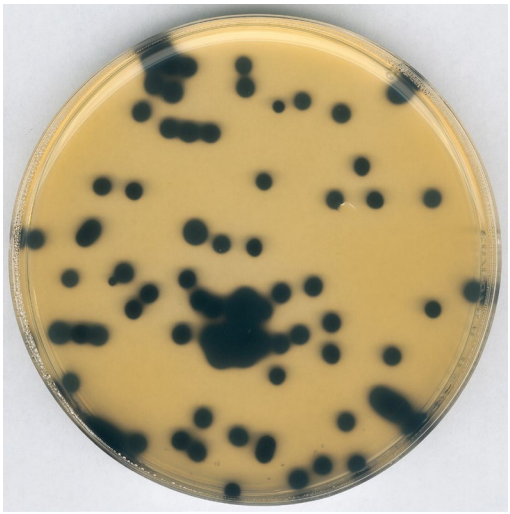
Test method: Performance testing of solid culture media - Quantitative and qualitative method (poured plate)

Function	Control strains	Incubation	Reference medium	Method of control	Expected results	Specified by
Productivity	*Clostridium perfringens* ATCC® 13124™ [WDCM 00007]	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	TSC agar, batch already validated	Quantitative, pour plate technique	Recovery ≥ 70 % black colonies	EN ISO 15213-2:2023 and EN ISO/TS 15213-3:2024
	Clostridium perfringens ATCC® 12916™ [WDCM 00080]					
	Clostridium perfringens ATCC® 10543™ [WDCM 00174]					
Selectivity	*Bacillus spizizenii* (formerly *Bacillus subtilis*) ATCC® 6633™ [WDCM 00003]	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	–	Qualitative, pour plate technique	Total inhibition	

Test method: Performance testing of solid culture media - Quantitative and qualitative method (membrane filtration)

Function	Control strains	Incubation	Reference medium	Method of control	Expected results	Specified by
Productivity	*Clostridium perfringens* ATCC® 13124™ [WDCM 00007]	(21 ± 3) h/ (44 ± 1) °C anaerobic atmosphere	TSC agar, batch already validated	Quantitative, membrane filtration technique	Recovery ≥ 70 % black colonies	EN ISO 11133: 2014/ Amd 1: 2018
	Clostridium perfringens ATCC® 12916™ [WDCM 00080]					
	Clostridium perfringens ATCC® 10543™ [WDCM 00174]					
Selectivity	*Bacillus spizizenii* (formerly *Bacillus subtilis*) ATCC® 6633™ [WDCM 00003]	(21 ± 3) h/ (44 ± 1) °C anaerobic atmosphere	–	Qualitative, membrane filtration technique	Total inhibition	

Please refer to the actual batch related Certificate of Analysis.
A recovery rate of 70 % is equivalent to a productivity rate of 0.7.
The filter type used for membrane filtration is made of mixed cellulose esters (MCE), pore size 0,45 µm, batch already validated according to EN ISO 7704:2023.



Clostridium perfringens WDCM 00007
in GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base)
acc. ISO 15213, ISO 14189 and FDA-BAM

Literature

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique. ISO 15213-2:2023.

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 3: Detection of *Clostridium perfringens*. EN ISO/TS 15213-3:2024.

EN ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of *Clostridium perfringens* — Colony-count technique. EN ISO 7937:2004 (withdrawn, revised by EN ISO 15213-2:2023).

EN ISO International Standardisation Organisation. Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration. EN ISO 14189:2013.

EN ISO International Standardisation Organisation. Water quality — Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) — Part 2: Method by membrane filtration. ISO 6461-2:1986 resp. EN 26461-2:1993.

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/Amd 1:2018/Amd 2:2020.

EN ISO International Standardisation Organisation. Water quality — Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods. EN ISO 7704:2023.

APHA (2015) Chapter No. 33: *Clostridium perfringens.* 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.

AOAC (2023): Official Method 976.30 *Clostridium perfringens* in Foods: Microbiological Method. AOAC International, Rockville, MD, USA.

FDA-BAM (2001): Chapter No. 16: *Clostridium perfringens*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2001): Media Index for BAM - BAM Media M169: Tryptose-Sulfite-Cycloserine (TSC) 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: Food microbiological examination: Examination of *Clostridium perfringens*. GB 4789.13-2012.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Tryptose Sulfite Cycloserine (TSC) agar (without egg yolk). In: Handbook of Culture Media for Food and Water Microbiology, pp. 784-786. Royal Society of Chemistry, Cambridge, UK.

Eisgruber, H., Schalch, B., Sperner, B., and Stolle, A. (2000): Comparison of four routine methods for the confirmation of *Clostridium perfringens* in food. Int. J. Food Microbiol. **57**: 135–140.

Eisgruber, H., Geppert, P., Sperner, B., and Stolle, A. (2003): Evaluation of different methods for the detection of *Clostridium perfringens* phosphatases. Int. J. Food Microbiol. **82**: 81– 86.

Fischer, M., Zhu, S. and de Ree, E. (2012): Culture media for the detection and enumeration of *Clostridia* in food. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 66-89. Royal Society of Chemistry, Cambridge, UK.

Hauschild, A.H.W., and Hilsheimer, R. (1974): Evaluation and modifications of media for enumeration of *Clostridium perfringens*. Appl. Microbiol. **27**: 78-82.

Hauschild, A.H.W., and Hilsheimer, R. (1974): Enumeration of food-borne *Clostridium perfringens* in egg yolk-free tryptose-sulfite-cycloserine agar. Appl. Microbiol. **27**: 521-526.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. Tryptose Sulfite Cycloserine (TSC) Agar Base w/wo Polymyxin and Kanamycin. pp. 806 – 808. Williams & Wilkins, Baltimore, MD, USA.

Sartory, D.P. (1986): Membrane filtration enumeration of faecal clostridia and *Clostridium perfringens* in water. Water Res. **20**: 1255–1260.

Sartory, D.P., Waldock, R., Davies, C.E., and Field, A.M. (2006): Evaluation of acid phosphatase as a confirmation test for *Clostridium perfringens* isolated from water. Lett. Appl. Microbiol. **42**: 418–424.

Shahidi, S.A., and Ferguson, A.R. (1971): New quantitative, qualitative and confirmatory media for rapid analysis for *Clostridium perfringens*. Appl. Microbiol. **21**: 500-506.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM	1.11972.0500	500 g
D-Cycloserine	C30020	1 g, 5 g, 25 g
Polymyxin B sulfate salt	P4932	1000000 units
Kanamycin sulfate	K1377	1 g, 5 g, 25 g
Clostridium perfringens selective supplement (contains D-Cycloserine and MUP)	1.00888.0010	10 x 1 vial
GranuCult® prime Iron Sulfite Agar acc. ISO 15213-1	1.10864.0500	500 g
GranuCult® prime Columbia Agar (base) acc. ISO 10272 and EP/USP/JP	1.00214.0500	500 g
GranuCult® prime SIM (Sulfite Indole Motility) Agar acc. ISO 15213	1.05470.0500	500 g
Bactident® Indole (KOVÁCS Indole reagent) acc. ISO and FDA-BAM	1.11350.0001	1 x 30 ml
KOVÁCS Indole reagent acc. ISO and FDA-BAM	1.09293.0100	100 ml
GranuCult® prime Tryptic Soy agar (TSA) acc. EP, USP, JP, ISO and FDA-BAM	1.05458.0500	500 g
GranuCult® prime Brain Heart Infusion (BHI) agar acc. FDA-BAM	1.03870.0500	500 g
Anaerocult® P Reagent for the generation of an anaerobic atmosphere for one Petri dish	1.32382.0001	25 x 1 set
Anaerocult® A mini Gas generator system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.32369.0001	25 x 1 set

Anaerocult® A Reagent for the generation of an anaerobic atmosphere in an anaerobic jar	1.32381.0001	10 x 1 piece
Anaerotest® Test stripes for the detection of an anaerobic atmosphere	1.32371.0001	50 test stripes
Anaerobic jar 2,5 l-volume	1.13681.0001	1 unit