Technical Data Sheet

GranuCult™
TSC (Tryptose Sulfite Cycloserine) Agar (Base)
acc. ISO 7937 and ISO 14189

Ordering number: 1.11972.0500

For isolation and differentiation of *Clostridium perfringens* from food and animal feed, water and other materials.

This culture medium complies with the specifications given by EN ISO 7937, ISO 14189 and APHA.

When supplemented with polymyxin B and kanamycin, the medium complies with the formulation given by SHAHIDI and FERGUSON (1971).

TSC agar with MUP can be prepared using Clostridium perfringens Supplement (article number 1.00888.0001) to detect acid phosphatase by its fluorescence under UV light.

Mode of Action

The superior nutrient base provides optimal conditions for the development of clostridia. Colonies producing hydrogen sulfide are characterized by blackening due to the reaction with sulfite and iron salt.

In TSC agar, cycloserine inhibits the accompanying bacterial flora and may cause the colonies which develop to remain smaller. It also reduces a diffuse and thus disturbing blackening around the *Clostridium perfringens* colonies.

TSC agar with MUP contains in addition to cycloserine 4-Methylumbelliferylphosphate (MUP), a fluorogenic substrate for the alcaline and acid phosphatase. The acid phopshatase is a high specific indicator for Clostridium perfringens. The acid phosphatase splits the fluorogenic substrate MUP forming 4-methylumbelliferone which can be identified by its fluorescence in long wave UV light. Thus a strong suggestion for the presence of *Clostridium perfringens* can be obtained.

SFP agar contains polymyxin B and kanamycin as selective inhibitors of accompanying flora. It is slightly less selective than TSC Agar.

Typical Composition

Specified by EN ISO 7937 and ISO 14189		GranuCult™ TSC agar (base) acc. ISO 7937 and ISO 14189	
Enzymatic digest of casein	15 g/l	Enzymatic digest of casein	15 g/l
Yeast extract	5 g/l	Yeast Extract	5 g/l
Enzymatic digest of soya	5 g/l	Enzymatic digest of soya	5 g/l
Sodium disulfite	1 g/l	Sodium disulfite	1 g/l
Iron(III)ammonium citrate	1 g/l	Ammonium iron(III) citrate	1 g/l
Agar	9-18 g/l	Agar-agar*	12 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	7.6 ± 0.2	pH at 25 °C	7.6 ± 0.2
Supplement to be added after autoclaving:			
D-Cycloserine	0.4 g/l	D-Cycloserine	0.4 g/l

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

Preparation

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 45-50 °C mix in 0.4 g/l Cycloserine for TSC-Agar or 3 mg/l Polymyxin sulfate and 12 mg/l Kanamycin sulfate for SFP-Agar as filter-sterilized solutions.

For the preparation of TSC agar with MUP, add the dissolved content of 2 vials Clostridium perfringens Supplement (article number 1.00888.0001) after autoclaving to the TSC agar base.
The prepared medium is clear and brown.

Experimental Procedure and Evaluation

Following the procedure for direct enumeration given by EN ISO 7937, inoculate TSC agar plates by means of a pipette 0.1 ml of the intial suspension or decimal dilution on the plate. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dry.

Incubate at 36-38 °C for 20-24 h under anaerobic conditions (e.g. using Anaerocult® A, Anaerocult® A mini, or Anaerocult® P in an anaerobic jar). Longer incubation may result in excess blackening of the plates.

Count the colonies on each plate and follow for confirmation the procedure e.g. given by EN ISO 7937.

Following the procedure for direct enumeration using TSC agar with MUP, inoculate by the pour plate technique only. Incubate at 40-48 °C for 20-24 h under anaerobic conditions (e.g. using Anaerocult® A, Anaerocult® A mini, or Anaerocult® P in an anaerobic jar).

Fluorescence can be detected with an UV lamp; light blue fluorescencing black colonies indicate *Clostridium perfringens* caused by phosphatase reaction with the methylumbelliferyl phosphate substrate.

Following the procedure for direct enumeration given by ISO 14289, inoculate TSC agar using the membrane filtration technique.

Incubate at 43-45 °C for 18-24 h inverted to avoid interference with condensing water under anaerobic conditions (e.g. using Anaerocult® A, Anaerocult® A mini, or Anaerocult® P in an anaerobic jar).

After incubation, enumerate the presumptive *Clostridium perfringens* by counting all colonies which show black or grey to yellow brown staining, even if the color is faint, of the TSC medium when viewed from either above or below the membrane filter.

Since the black color of the colonies rapidly fades and finally disappear, the plates have to be counted within 30 min after completion of the anaerobic incubation. If more anaerobic jars are used, the plates should be checked jar by jar or in portions if the incubation was performed in an anaerobic incubator.

For confirmation follow the procedure e.g. given by ISO 14289.

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 ISO 14189, self-prepared plates should be used as fresh as possible on the same day.

If storage of the prepared plates is inevitable, store the plates under anaerobic conditions at +2 °c to +8 °C and use them within 7 days.

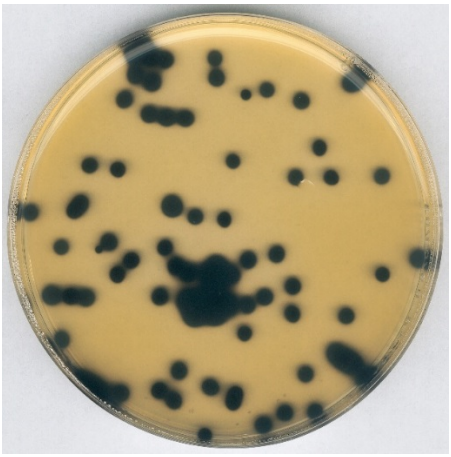
Quality Control

GranuCult™ TSC agar (base) acc. ISO 7937 and ISO 14189 is tested with supplementation of Clostridium perfringens Supplement (article number 1.00888.0001).

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Clostridium perfringens* ATCC® 13124	18-22 h at 36-38 °C anaerobic	GranuCult™ TSC agar	Quantitative	Recovery ≥ 70 %, blackening of the colonies and fluorescence under UV light
	Clostridium perfringens ATCC® 12916				
	Clostridium perfringens ATCC® 10543				
Selectivity	*Clostridium tetani* ATCC® 19406	18-22 h at 36-38 °C anaerobic	-	Qualitative	None to fair growth, no blackening of the colonies
	Clostridium novyi ATCC® 17861				
	Bacillus cereus ATCC® 11778				None to fair growth, no blackening of the colonies
	Pseudomonas aeruginosa ATCC® 27853				None to poor growth, no blackening of the 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 70 % is equivalent to a productivity value of 0.7.



Clostridium perfringens ATCC® 13124

Literature

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

Araujo. M., Sueiro, R.A., Gómez, M.J. and Garrido, M.L. (2001): Enumeration of *Clostridium perfringens* spores in ground water samples: comparison of six culture media. J. Microbiol. Methods. **57**: 175–180.

Burger. J.S., Nupen, E.M. and Grabow, W.O.K. (1984): Evaluation of four growth media for membrane filtration counting of *Clostridium perfringens*. Water S.A. **10**: 185–188.

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

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Clostridium perfringens - Colony-count technique. EN ISO 7932:2004.

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

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

Orth, D.S. (1977): Comparison of sulfite-polymyxin-sulfadiazine medium and tryptose-sulfite-cycloserine medium without eggyolk for recovering *Clostridium perfringens*. Appl. Envir. Microbiol. **33**: 986-988.

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

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™ TSC (Tryptose Sulfite Cycloserine) Agar (Base) acc. ISO 7937 and ISO 14189	1.11972.0500	500 g
D-Cycloserine powder	C6880-1G	1.5 g
D-Cycloserine powder	C6880-5G	5 g
Clostridium Perfringens Supplement (containing Cycloserine and MUP)	1.00888.0010	10 vials
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Plate basket	1.07040.0001	1 ea
EZ-PAK Filters 0,45µm 47 mm white, gridded	EZHAWG474	4 bands of 150 filters
S-PAK mixed cellulose ester filter 0.45µm 47 mm white, gridded	HAWG047S6	600 individually sealed filters, sterile
UV Lamp (366 nm)	1.13203.0001	1 ea

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