Tryptose sulfite cycloserine (TSC) agar (ISO) Code 84636.0500

Also known as
TSC agar, TS(C) agar

Intended use
For the enumeration of *Clostridium perfringens* in food and animal feeding stuffs (ISO 7937, ISO 15213).

Formula* - Composition in g/L
Enzymatic digest of casein..... 15.0
Soy peptone..... 5.0
Yeast extract..... 5.0
Disodium disulphite anhydrous..... 1.0
Ammonium iron(III) citrate..... 1.0
Agar..... 15.0

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.6 ± 0.2 at 25 °C.

Instructions for preparation
Dissolve 21 g in 500 ml of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes and cool to 45-50 °C. Dissolve the contents of one vial of D-Cycloserine Supplement (Art.:84734.0001) with 5 ml of purified water and add to the cooled medium. Mix well and distribute into sterile Petri dishes.
For the preparation of Sulfite iron agar use the medium base without Cycloserine Supplement.

Principle of the method and general information
Tryptose sulfite cycloserine (TSC) agar is prepared according to a modification of the formula proposed by Harmon and Kauttar and meets the requirements of the formulations recommended by ISO 7937 and ISO 15213 for the enumeration of *C.perfringens* and sulphite reducing bacteria growing under anaerobic conditions, respectively, in food and animal feeding stuffs. The medium utilises the selective inhibitory properties of D-cycloserine and the indicator system involving sulphite and ferric iron. Most unwantend organisms are suppressed, while *C.perfringens* and related species will grow reducing the sulphite and forming black colonies, due to the production of ferrous sulphide.
Originally the medium was proposed with the addition of egg yolk, but the egg yolk-free modification of Hauschild and Hilsheimer is more convenient.
The medium without D-cycloserine corresponds to sulphite iron formulation proposed by ISO 6461-2 for the enumeration of sulphite reducing anaerobes in water by MF technique.

Instruction for use
For laboratory use only.
For the enumeration of *C. perfringens,* ISO 7937 recommends the technique summarized below.
- Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.(e.g. Maximum Recovery Diluent Art. N° 84617.500)
- Transfer by means of sterile pipettes 1 ml of the test sample (if liquid) or 1 ml of the initial suspension and 1 ml of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
- Pour 15 – 20 ml of TSC Agar into each dish and mix well with the inoculum.
- When the medium has solidified add an over layer of 10 ml of the same TSC Agar.
- Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37 °C for 20 hours. Longer incubation may result in excess blackening along the bottom rim of the plates.

VWR International bvba/sprl
Haasrode Research park Zone 3
Geldenaaksebaan 464
b-3001 Leuven Tel +32 (0) 16 385 011

Revision: TS-846360500.docx rev 0 - 5-2013

- Count the black colonies on the plates containing between 15 and 150 characteristic colonies. If parts of the plates are completely blackened count the colonies at the next higher dilution even their number may be less than 15.

Limitation

- It is recommended that biochemical and/or serological tests be performed on pure culture for complete identification.

Quality Control

Physical characteristics:

Appearance of powder	Beige, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Yellow, limpid
pH (25°C)	7.6 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity rate
C. perfringens ATCC 13124	37 °C / 20 h AN	QT	Black colonies with black halo	PR ≥ 0.7
C. perfringens ATCC 12916	37 °C / 20 h AN	QT	Black colonies with black halo	PR ≥ 0.7
E. coli ATCC 25922	37 °C / 20 h AN	MM	Totally inhibited	
P.aeruginosa ATCC 27853	37 °C / 20 h AN	MM	Partially inhibited	
P.mirabilis ATCC 10005	37 °C / 20 h AN	MM	Partially inhibited	
TS (C) without D-cycloserine				
C. perfringens ATCC 13124	37 °C / 20 h AN	QT	Black colonies with black halo	PR ≥ 0.7
C. perfringens ATCC 12916	37 °C / 20 h AN	QT	Black colonies with black halo	PR ≥ 0.7
E. coli ATCC 25922	37 °C / 20 h AN	EC	Good growth colourless colonies	

Notes

Medium supplementation: D-Cycloserine Supplement (REF 84734.0001)
PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch
Incubation atmosphere AE:aerobic incubation
Inoculation method QT : quantitative surface plating method; EC: semi-quantitative, ecometric technique; MM: modified Miles-Misra surface drop method
Microbiological characteristics tested in accordance to ISO/TS 11133-2
ATCC is a registered trade mark of American Type Culture Collection

References

- Baird, R.M. Corry, J.E.L and G.D.W. Curtis Pharmacopoeia of Culture Media. 1987. Int. J. Food Microbiol. 5, 278-279
- Harmon, S. M., and D. A. Kauttar, J. T. Peeler. 1971. Appl. Microbiol. 22:688-692.
- Hauschild, A.H.W. and R. Hilsheimer. 1974. Appl. Microbiol. 27, 78-82
- ISO 15213:2003 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of sulphite reducing bacteria growing under anaerobic conditions.
- ISO 6461-2:1986 Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 2: Method by membrane filtration
- ISO 7937:2004 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of *Clostridium perfringens* -- Colony-count technique

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10 ºC to 30ºC and <60% RH).

Ordering information

Dehydrated medium:

84636.0500 Tryptose sulfite cycloserine (TSC) agar (ISO)	Bottle of 500 g

Supplement:

84734.0001 D-Cycloserine Supplement	10 vials each for 500 ml of complete medium