

# TECHNICAL DATA SHEET

Article No. 8032

TSC Agar (Tryptose Sulphite Cycloserine Agar) Base

## SYNONYMS

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## SPECIFICATION

Solid medium for the isolation and differentiation of *Clostridium perfringens*. DIN 10165, ISO 7937, ISO 14189

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## FORMULA\* IN G/L

Tryptone	15.00
Soy peptone	5.00
Yeast extract	5.00
Sodium disulphite	1.00
Ammonium iron(III) citrate	1.00
Agar	18.00

Final pH 7.6 ±0.2 at 25 °C

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

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## DIRECTIONS

Suspend 45 g of powder in 1 l of distilled water and let it soak. Heat to boiling and distribute into suitable containers, do not add more than 250 ml to each one. Sterilize by autoclaving at 121 °C for 10 minutes. Let it cool to 60 °C and add one vial D-Cycloserine Selective Supplement (Art. no. 9795) to every 250 ml of medium. If desired add Egg Yolk Sterile Emulsion (Art. no. 9578) in a concentration of 80 ml/l. Fluorogenic Supplement MUP may be used to identify *Clostridium perfringens* (Art. no. 9716). Mix well and pour into plates.

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## DESCRIPTION

The medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin, have been replaced by cycloserine. Cycloserine has been found more selective for *Clostridium perfringens*, and reduces the production of diffuse blackening. *Clostridium perfringens* is more resistant to cycloserine than to sulfadiazine, polymyxin and neomycin, hence reducing the dosage. The presence of sodium meta-bisulfite and ferric ammonium citrate allow three differential characteristics of this anaerobic species to be



verified with just one assay. These characteristics are sulfite reduction, growth at 46 °C and cycloserine resistance.

Cycloserine does not tolerate temperatures above 100 °C and its stability in a solution is variable. Therefore, it is advisable to prepare the exact number of plates that are going to be used.

A solution of cycloserine in phosphate buffer at pH 8.0 may be prepared (di-potassium phosphate 16.73 g/l and potassium phosphate 0.52 g/l) and if it is maintained refrigerated, can be used for approx. 5 days. This product, stored at -20 ±5 °C can be used within 4 weeks of preparation.

#### Available supplements

##### D-Cycloserine Selective Supplement (Art. no. 9795)

Vial contents:

Necessary amount for 250 ml of complete medium.

D-Cycloserine 100 mg

Distilled water (solvent)

##### Clostridium perfringens Supplement (Art. no. 9716)

Vial contents:

Necessary amount for 200 ml of complete medium.

D-Cycloserine 100 mg

4-Methylumbelliferyl phosphate 25 mg

Distilled water (solvent)

## TECHNIQUE

The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis. After incubation at 44-46 °C for 24 ±3h, proceed to enumerate the black colonies that appear in the plate.

## QUALITY CONTROL

- Incubation temperature: 44 ±1.0 °C
- Incubation time: 21 ± 3 h
- Inoculum: Practical range 100 ±20 CFU. Min. 50 CFU (productivity)/10<sup>4</sup>-10<sup>6</sup> CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
<i>Clostridium perfringens</i> ATCC® 10543	Productivity >0.50	Black colonies (Anaerobiosis)
<i>Clostridium perfringens</i> ATCC® 13124	Productivity >0.50	Black colonies (Anaerobiosis)
<i>Bacillus subtilis</i> ATCC® 6633	Inhibited	None

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## REFERENCES

- ATLAS, R.M., LC. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- DIN Standard 10165. Referenz Verfahren für Bestimmung von Clostridium perfringens. Fleisch und Fleischerzeugnissen.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. American Public Health Association. Washington.
- DIRECTIVA 2015/1787/UE de la Comisión por la que se modifica la Directiva 98/ 83/CE relativa a la calidad de las aguas destinadas al consumo humano (DO L260 de 7.10.2015 pg 6 y ss)
- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International Inc. Gaithersburg, MD.
- ISO 7937 (2004) Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for Enumeration of C. perfringens. Colony-count technique.
- ISO Norma 6461-2 (1986) Water Quality- Detection and enumeration of the spores of sulfite-reducing anaerobes (Clostridia)- Part 2: Method by Membrane Filtration.
- ISO 11133:2014. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 14189 (2013) Water quality. Enumeration of Clostridium perfringens — Method using membrane filtration
- SMITH, L.D. (1981) Clostridial Anaerobic Infections, in Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections. 6th ed. APHA. Washington.
- UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.

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## STORAGE

Keep tightly closed, away from light, in a dry place (4-30 °C).

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## SHELF LIFE

4 years from date of production

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updated: 17.03.2023

