

Scharlau Microbiology - Technical Data

Product: D-Cycloserine Selective Supplement (200 mg)

Specification

A sterile selective supplement used for isolation and presumptive identification of *Clostridium perfringens*, according to ISO 7937 and ISO 14189, and other regulations.

Presentation				
10 vials Vial with: 6 ± 0.1 g	Packaging Details 23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.		Shelf Life 49 months	Storage 2-25 °C
Composition				
Compositon (g/vial)		NOTE: each vial is sufficient to supplement 500 ml of medium		
D-Cycloserine	0.200	TSC Agar Base.		
Reconstitute the original freeze-dried vial by adding				
Sterile Distilled Water	6 ml			

Description /Technique

Description:

D-cycloserine selective supplement is added to TSC Agar in order to obtain a final selective medium which has the advantage to simplify the counting of plates with high numbers of colonies because smaller colonies of *C.perfringrens* are formed. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction made by *Clostridium perfringens* spp. that produce black colonies in TSC agar.

Technique:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial in aseptic conditions and add it to 500 ml of melted Agar base cooled to 50°C.

Do not overheat once suplemented.

Pour the complete medium into Petri dishes (or tubes) and, once solidified on a flat surface, spread the plates either by streaking by spiral method or dilution banc.

Incubate the plates in anaerobic atmosphere at $35 \pm 2^{\circ}$ C for 24-24h. To obtain a more selective medium, incubated at 44 ° C ± 1. Incubation times longer than those mentioned above or different incubation temperatures may be requied depending on the sample or the specifications.

After incubation, count all the colonies that have appeared onto the surface of the agar.

C.perfingrens grows in black colonies, due to the iron sulfide precipitation.

Presumptive isolation of *Clostridium perfringens* must be confirmed by further microbiological and biochemical tests.



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Quality control

Physical/Chemical control Color : White-Gray

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosis. Incubation at 44 ± 1 °C during $21 \pm 3h$.

Microorganism

Clostridium perfringens ATCC[®] 13124, WDCM 00007, NCTC[®] 8237 Clostridium perfringens ATCC[®] 10543, WDCM 00174 Bacillus subtilis ATCC[®] 6633, WDCM 00003

Sterility control

Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate. Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH. Check at 7 days after incubation in same conditions.

Bibliography

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• 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)

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· 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/ Adm 1:2018. 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.

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Growth

Good - black colonies Good - black colonies Inhibited