Reference: 06-743LYO1 **Scharlau Microbiology - Technical Data**

Product: **D-Cycloserine Selective Supplement**

Specification

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

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 ºC

Composition

Compositon (g/vial)

D-Cycloserine..... 0.040

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....6 ml

NOTE : Each vial is sufficient to supplement 100 ml of medium Base: TSC Agar Base

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 with 6 ml of sterile diluent in aseptic conditions and add it to 100 ml of melted Agar base cooled to 50ºC.
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 ± 2ºC for 20-24h. 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.

Quality control

Physical/Chemical control

Color : White-Gray

Microbiological control

Add to 100 ml TSC Base

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

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

Distribute the complete medium, cooled to 50ºC, into suitable containers

Incubate according instructions for complete medium indicated in COMPOSITION.

Anaerobiosis. Incubation at 44 ± 1 ºC during 21 ± 3h.

Microorganism	Growth
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good - black colonies
Clostridium perfringens ATCC® 10543, WDCM 00174	Good - black colonies
Bacillus subtilis ATCC® 6633, WDCM 00003	Inhibited

A double layer with TSC agar favors the observation of the blackening of the SH2 (+) strains.

Sterility control

Add 5 ml of the sample to:
100 ml TSB and 100 ml Thioglycollate.
Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.

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