

D-cycloserine Supplement

*For microbiological control only*Enumeration of *Clostridium perfringens*.

SUMMARY AND EXPLANATION

The D-cycloserine supplement is used as additive for T.S.C Agar (or TSC water base). The addition of this chemical increases the selectivity of the medium towards *Clostridium perfringens*.

PRINCIPLE

The addition of 0.4 g/l of D-cycloserine enables a TSC agar to be obtained for the enumeration of *Clostridium perfringens*, according to the recommendations of the standard ISO 7937(1) and NF T90-415(3). French standard NF V 08-061(2) recommends to add D-cycloserine for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.

CONTENT OF THE KIT

Lyophilised supplement

REF AEB184002/10

Pack of 10 Q.S.P 500 ml (freeze-dried)

COMPOSITION

Theoretical formula in g/l after addition of a supplement bottle to the base

This medium can be adjusted and/or supplemented according to the performance criteria required:

D-cycloserine.....0, 4

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagents:

- T.S.C base agar
- T.S.C water base agar

Material:

- Sterile or aseptic Petri plates.
- Water baths.
- Anaerobiosis generators, e.g.: GENbox anaer (Ref. 96 124) or GENbag anaer (Ref. 45 534).
- Jars, e.g.: GENbox Jar 2.5L (Ref. 96 127).
- Bacteriology incubator.

Or

- Thermoregulated chambers with a controlled atmosphere.

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLS[®] M29-A, Protection of Laboratory Workers From occupationally Acquired Infections; Approved Guideline – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents past the expiry date.

- Do not use bottles/tubes which show signs of contamination.
- Before use, make sure the bottle caps are intact.
- The medium should be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the bottles in their box at 2-8°C until the expiry date.**
- Once regenerated the supplement can be kept up to 12 hours at 2-8°C.
- Fraction the supplement into tubes and freeze them at – 20°C to keep them up to 2 months.

SPECIMENS

Follow the recommendations in the current standards to perform collection and preparation.

INSTRUCTIONS FOR USE

1. Collect aseptically the content of a bottle by 5 ml of sterile purified water
 2. Agitate until complete dissolution.
 3. Add the content of the 500ml T.S.C or T.S.C water base agar bottle previously regenerated and maintained at 44-47°C or 0, 2 ml in a tube containing 20 ml of medium.
 4. Homogenize the agar thus added with D-cycloserine and use it at once.
 5. Inoculate according to the method described in the chosen reference standard (1, 2,3).
- As a general rule, dispense 1 ml of test sample or 1 ml of stock solution into a sterile Petri dish. Add approximately 15 ml of T.S.C Agar maintained at 44-47°C and mix thoroughly. Leave to set on a flat surface. Add 5 to 10 ml of the same agar, maintained at 44-47°C and leave to set.
6. Incubate for 20 ± 2 hours under anaerobic conditions at 37 ± 1°C or 46 ± 1°C. The choice of the incubation temperature is the responsibility of the user, according to procedure and current regulations.

Notes:

- The time between inoculation of the Petri dishes and addition of the agar medium must not exceed 15 minutes.

READING AND INTERPRETATION

- After incubation, count the number of characteristic black colonies.
- For the interpretation of results, refer to the chosen reference standard (1, 2,3).

QUALITY CONTROL

D-cycloserine supplement is designed and developed to meet the strictest quality requirements.

The quality control results of strains tested batch by batch are given on the quality control certificate which is available on request.

LIMITATIONS OF THE METHOD

- Once added with D-cycloserine, T.S.C and T.S.C water base agars therefore obtained have to be used at once.

- Certain *Clostridium bifermentans*, *C. sordelli*, *C. tetani* and *C. sporogenes* strains grow on TSC agar and produce characteristic colonies.
- Given the wide variety of specimens tested, it is the responsibility of the user to validate this medium in its specific application.

WASTE DISPOSAL









Unused reagents may be considered as non hazardous waste and disposed of accordingly. Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. Norme ISO 7937 (V 08-019) – Microbiology of food and animal feeding stuffs. Horizontal method for enumeration of *Clostridium perfringens*. Colony-count technique. April 1997. ISSN 0335-3931.
2. Norme NF V 08-061 – Microbiologie des aliments . Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies. Méthode de routine.
3. AFNOR NF T90-415. Essais des eaux - Recherche et dénombrement des spores de bactéries anaérobies sulfito-réductrices et de *Clostridium* sulfito-réducteurs. Méthode générale par incorporation en gélose en tubes profonds

INDEX OF SYMBOLS

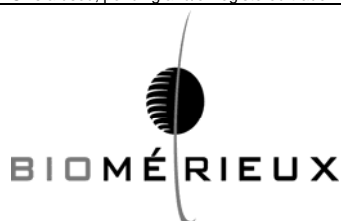
Symbol	Meaning
	Catalogue number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Keep away from light

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