REF 42 619

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D-cycloserine Supplement (CYCLO)

For microbiological control only

Enumeration of *Clostridium perfringens* in food products.

SUMMARY AND EXPLANATION

D-cycloserine Supplement is added to Iron Sulfite agar (Ref. 42 603) to obtain Cycloserine Sulfite (SC) agar, previously called Tryptose Sulfite Cycloserine (TSC), for the enumeration of *Clostridium perfringens*, according to the recommendations of the standards NF EN 13401, ISO 7937 and routine standards NF V 08-056 and NF V 08-061 (1, 2, 3, 4).

PRINCIPLE

The addition of 0.4 g/l of D-cycloserine to Iron Sulfite agar enables a selective medium to be obtained (SC) which inhibits the growth of most *Clostridium* other than *C. perfringens* when incubated at 35°C or 37°C in anaerobic conditions.
The presence of black colonies (H_2S +) is a strong indication of *C. perfringens.*

CONTENT OF THE KIT

	Ready-to-use medium
REF 42 619	6 x 2 ml bottles (lyophilized) + 1 package insert

COMPOSITION

Theoretical formula in g/l after addition of one bottle of supplement to 200 ml of agar.

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

D-cycloserine	0.4

This reagent contains inert compounds made from animal products of bovine origin.

REAGENT AND MATERIAL REQUIRED BUT NOT PROVIDED

Reagent :

- Iron Sulfite agar (Ref. 42 603)

Material :

- Sterile Petri dishes.
- Water-baths.
- Bacteriology incubators.
- 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).

or

- Thermoregulated chambers with a controlled atmosphere.

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- 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 "NCCLS M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline - Current Revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH, Latest Edition", or to the regulations currently in use in each country.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents past the expiry date.
- 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.**
- **After reconstitution, D-cycloserine can be stored for 1 hour at room temperature.**

SPECIMENS

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

INSTRUCTIONS FOR USE

1. Aseptically reconstitute the contents of one bottle with 2 ml of sterile demineralized water.
2. Shake until completely dissolved.
3. Add the contents of the bottle to 200 ml of Iron Sulfite agar previously melted and maintained at 50°C ± 3°C (refer to the Instructions for use in the Iron Sulfite agar technical sheet).
4. Homogenize the agar which has been supplemented with D-cycloserine and use immediately.
5. Inoculate according to the method described in the reference standard for the enumeration of *Clostridium perfringens* (2) :
   As a general rule, dispense 1 ml of test sample or 1 ml of stock solution into a sterile Petri dish.
   Immediately add approximately 15 ml of SC agar maintained at 50 ± 3°C and mix thoroughly.
   Leave to set on a flat surface.
   Add 5 to 10 ml of the same agar, maintained at 50 ± 3°C and leave to set.
   Incubate for 20 ± 2 hours, in anaerobic conditions, at 35 ± 1°C or 37 ± 1°C for the enumeration of *Clostridium perfringens*. The user is responsible for choosing the appropriate incubation temperature for the intended use, in accordance with current standards.
   The tests are performed twice for the chosen serial dilutions.

Note:

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.
- Only take into account Petri dishes containing less than 150 characteristic colonies.
- For the interpretation of results, refer to the reference standard (2).

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 SC agar has been supplemented with D-cycloserine, it must be used immediately.
- Certain strains of *Clostridium bifermentans, C. sordelli*, *C. tetani* and *C. sporogenes* grow on SC agar and produce characteristic colonies.
- Iron Sulfite agar is maintained in a liquid state at a temperature of 50 ± 3°C. This temperature does not destroy *C. perfringens*. A lower temperature could cause the agar to partially solidify.
- Before addition of D-cycloserine, the Iron Sulfite agar must be maintained for a minimum of 30 minutes and a maximum of 4 hours at 50°± 3°C.
- Given the wide variety of specimens tested, it is the responsibility of the user to validate this medium in its specific application.

WASTE DISPOSAL

Dispose of used or unused 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. NF EN 13401 – Horizontal method for enumeration of *Clostridium perfringens*. Colony-count technique. January 2003. ISSN 0335-3931.
3. Norme NF V 08-056 – Microbiologie alimentaire. Dénombrement de *Clostridium perfringens* par comptage des colonies à 37°C. Méthode de routine. Avril 1994. ISSN 0335-3931
4. Norme XP V 08-061 –Microbiologie des aliments . Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies. Méthode de routine. Octobre 1996. ISSN 0335-3931
5. Culture Media for Food Microbiology, J.E.L. Cory et al. (Eds.) – 1995 – Elsevier Science 25-33 et 458-460.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use

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bioMérieux SA
376 Chemin de l'Orme
69280 Marcy-l'Etoile - France

673 620 399 RCS LYON
Tél. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com