Reference: 06-125LYO1 **Scharlau Microbiology - Technical Data**

Product: **m-CP Selective Supplement**

Specification

Sterile selective supplement used for the isolation and identification of *Clostridium perfringens* .

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	0.2000
Polymyxin B sulphate	0.0125
3-Indoxyl-ß-D-Glucopyranoside	0.0300
Phenolphtalein bi-phosphate	0.0500
Iron III chloride	0.0450

Reconstitute the original freeze-dried with:

Sterile Distilled Water	10 ml

NOTE : Each vial is sufficient to supplement 500ml of m-CP Agar base.

Description /Technique

Description:

Membrane Clostridium Perfringens (m-CP) selective supplement is a mix of chromogenic substrates and antibiotics that is added to m-CP medium base in order to obtain a selective medium for the presumptive identification of Clostridium perfringens from water samples.
In m-CP Medium lack of b-D-glucosidase activity (an enzyme involved in cellobiose fermentation), fermentation of sucrose and production of acid phosphatase are used to differentiate presumptive *Clostridium perfringens* colonies from other *Clostridium spp*.
Lack of b-D glucosidase activity means that *Clostridium perfringens* does not cleave the chromogen, indoxyl b-D glucoside, in the medium. Furthermore, as the organisms ferment the sucrose in the medium, reducing the pH, bromocresol purple changes from purple to yellow. This results in characteristic opaque yellow *Clostridium perfringens* colonies.
Most other *Clostridium spp.* will appear as either purple colonies, due to the lack of sucrose fermentation, or blue/green colonies where the organism is still cleaving Indoxyl b-D glucoside and also fermenting sucrose.
D-cycloserine, polymyxin B and incubation at 44°C inhibit the growth of background flora such as Gram-negative organisms and staphylococci.
m-CP Medium has now been recommended in European Council Directive 98/83/EC for testing the quality of water intended for human consumption

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 10 ml of the sterile diluent in aseptic conditions, shake a few seconds, and add it to 500 ml of the medium base cooled to 50ºC.
Do not overheat once supplemented.
Pour the complete medium into Petri dishes and, once solidified on a flat surface, inoculate it with a 0.45 mm pore membrane with which was filtered the sample.
Incubate the plates in anaerobically at 44ºC for 20-24h.
Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications.
Each laboratory must evaluate the results according to their specifications.
Presumptive positive *Clostridium perfringens* colonies can be further tested for acid phosphatase activity by exposure to ammonium hydroxide vapour for 20 to 30 seconds. *Clostridium perfringens* colonies turn pink or red as phenolphthalein diphosphate is cleaved by acid phosphatase. No colour change will be seen with colonies of organisms that do not posses acid phosphatase. It is important this further test is carried out as there are a very small number of non-perfringens clostridia that produce yellow colonies. However, these colonies will remain yellow after exposure to ammonium hydroxide as they are acid phosphatase negative.

Quality control

Physical/Chemical control

Color : Red

Microbiological control

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

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

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

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

Microorganism	Growth
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good - opaque yellowish colonies
Clostridium perfringens ATCC® 10543, WDCM 00174	Good - opaque yellowish colonies
Clostridium bifermentans NCTC® 506	Good -Blue colonies
Escherichia coli ATCC® 8739, WDCM 00012	Inhibited

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.

Bibliography

· European Council (1997) Directive 12767/97 on the quality of water destined for human consumption. EC Bull. 16-12-1997.

. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.