12398 CP *ChromoSelect* Agar (Clostridium perfringens *ChromoSelect* Agar; CPC Agar; Clostridium perfringens Chromogen Agar)

A chromogenic agar is developed for enumeration and differentiation of *Clostridium* sp., in particular *Clostridium perfringens*, in aqueous samples. The medium is more reliable than m-CP and TSC agars and easier to handle.

Composition:

Ingredients	Grams/Litre
Tryptose (vegetable)	20.0
Yeast Extract	15.0
Soy peptone	5.0
Sucrose	3.0
L-Cysteine hydrochloride	1.0
Magnesium sulfate heptahydrate	0.1
Ammonium iron (III) citrate	0.2
Tris buffer	1.8
chromogenic mixture	1.73
Agar	15.0
Final pH 7.4 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance: slightly yellow to brown coloured, homogeneous, free flowing powder.
Gelling: firm
Colour and Clarity: yellow to brown coloured clear to slightly turbid gel forms in Petri plates.

Directions:
Suspend 31.42 g in 400 ml distilled water and adjust the volume to 500 ml. Boil to dissolve the medium completely. Cool to 50-55°C and add 1 vial of Perfringens T.S.C. Supplement (Cat. No. P9352) or 1 vials of M-CP selective Supplement I (Cat. No. 51962, for heavy contaminated waters). Dispense into Petri dishes (we recommend small ones).

Experimental Procedure:
The membrane-filter-technique is recommended. The type of membrane filter could affect the performance of the medium (recovery rate and colouration of colonies) [1]. Incubation: 24 ± 2 hours at 44°C under anaerobic conditions. After incubation, open the plate and incubate 1 h aerobically at 44°C. If the colonies of *C. perfringens* are already green, you do not need further incubation.

Principle and Interpretation:
CP *ChromoSelect* Agar is a new chromogenic media specially developed for the detection and enumeration of *Clostridium* sp., in particular *Clostridium perfringens* in aqueous samples.

To the common media, it has the advantage there is no diffusion in agar and no confirmation is needed. The green colonies are high specific for *C. perfringens*. Using mCP agar, *C. perfringens* colonies cannot be used for subcultur because of the use of Ammonia and mCP is too selective. The red colour of colonies, after the addition of ammonia, disappears and further confirmation is not possible.

TSC agar detects all SRC and is based on the reduction of sulphite to sulphide by the enzyme sulphite reductase. This is visible in the presence of iron as black colonies surrounded by a black halo in solid media. Another disadvantage of TSC is although in some cases, excessive blackening of the agar frustrated counting of the lower dilutions, even though low cell numbers were expected. If the contamination was too high, TSC did not consistently produce black colonies. When the medium becomes acidified, iron sulphide will no longer precipitate and disappears because of the oxygen, which produces false negative results.

CP *ChromoSelect* Agar contains a vegetable peptone meets the nutritional features from tryptose. Together with soy peptone and yeast extract it acts as a source of nitrogen, carbon, amino acids and vitamin B complex.
Sucrose is a fermentable sugar for *Clostridium perfringens* and L-Cysteine hydrochloride is a reducing agent, and lower the redox potential of the medium. Magnesium sulfate heptahydrate is a source of magnesium ions required in a variety of enzymatic reactions, while ammonium iron (III) citrate increased the fermentation reactions by providing important ions and substrates. The pH is stabilized by Tris buffer, *Cl. perfringens* is starting to be inhibited by a pH of 7.6. The chromogenic mixture contains enzyme substrates, inhibitors and different promoters to protects injured cells, to improve recovery rate and to enhance growth.

The addition of supplement with D-Cycloserine and Polymyxin B makes the medium inhibitory to accompanying non-clostridial flora and thus allows analysis of both vegetative cells and spores of Clostridium. Further selectivity is provided by incubation under anaerobic conditions at 44°C.

For further confirmation of *Clostridium perfringens* it is suggested to carry out following biochemical test:
Sulphite reduction, gram-reaction, sporulating rods, motility, reduction of nitrate, gelatin liquefaction, and lactose fermentation.

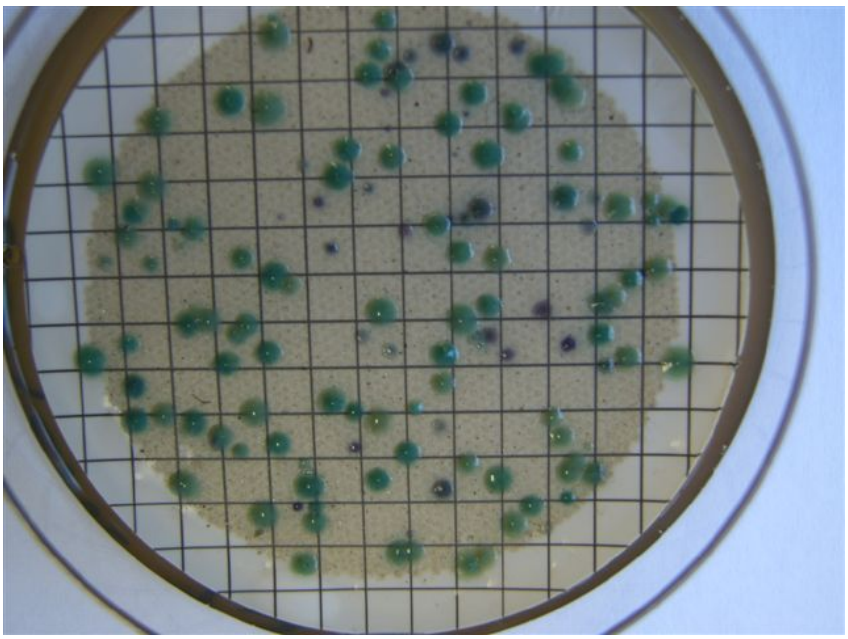
Cultural characteristics after 24 h anaerobic incubation at 44°C followed by 1-2 hours aerobic incubation at 44°C.

Organisms (ATCC)	Growth	Colony appearance
Clostridium perfringens (13124)	+++	green
Clostridium bifermentans (638)	+++*	dark blue with violet halo
Clostridium sporogenes (8534)	-	-
Clostridium sordelli (9714)	++	dark green with halo (change to red with Kovac's Reagent)
Enterococcus faecalis (29212)	++	violett
Escherichia coli (25922)	-	-
Pseudomonas aeruginosa (27853)		colourless
Staphylococcus aureus (25923)	-	-
Bacillus subtilis (6051)	-	-
Salmonella typhimurium (DSM 554)	++	violett

* Key: growth at 40°C but no growth at 44°C

References:

1. D.P. Sartory, M. Field, S.M. Curbishley, A.M. Pritchard, Evaluation of two media for the membrane filtration enumeration of Clostridium perfringens from water, Letters in Applied Microbiology, 27, 323-327 (1998)



Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.