Reference: 101274ZA **Technical Data Sheet**

Product: # Chromocult® Coliform Agar (ISO)

Specification

Solid, selective and differential culture medium for the detection and enumeration of total coliform and *E. coli* in water samples by the MF technique acc. to ISO 9308-1 standard.

Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 21 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14 ºC

Composition

Composition (g/l)	
Enzymatic digest of casein	1.00
Yeast extract	2.00
Sodium chloride	5.00
Sodium dihydrogen phosphate	2.20
Disodium hydrogen phosphate	2.70
Sodium pyruvate	1.00
L-Tryptophan	1.00
Sorbitol	1.00
Tergitol® 7	0.15
6-Chloro-3-indoxyl-ß-D-galactopyranoside	0.20
5-Bromo-4-chloro-3-indoxyl-ß-D-glucuronic acid	0.10
IPTG	0.10
Agar	10.0

Description /Technique

Description:

The combined action of peptone, pyruvate and sorbitol allow rapid colony growth in this phosphate buffered medium, which also permits simple recovery of sublethal thermally injured coliforms. Sodium chloride provides the correct osmotic environment necessary for growth.

The selectivity is attained, partially, by the Tergitol® 7, which inhibits the growth of Gram positive bacteria and some Gram negative without effecting the coliform bacteria. The colonial differentiation is due to the chromogenic mixture, composed of two enzyme substrates: 6-chloro-3-indoxyl-ß-D-galacto-pyranoside (Salmon®-GAL) and 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide (X-Glucuronide). The first one is cleaved by the characteristic enzyme found in coliforms, ß-D-galactosidase and gives a salmon-red colour to the coliform colonies. The second chromogenic substance is cleaved by the ß-D-glucuronidase enzyme characteristic of *E. coli* and turns the colonies of these bacteria a blue colour. *E. coli* has the two enzymes and cleaves both chromogenic substances giving dark blue to violet colonies. Total coliforms are the sum of *E. coli* colonies plus salmon-red colonies. The IPTG, enhances the reactions described above. Other Gram negative bacteria produce colourless colonies except some that possess glucuronidase activity (but not galactosidase) and they produce light blue to turquoise colonies.

To confirm the *E. coli* colonies in this medium a small amount of tryptophane is included verifying indol production: coat the blue-violet colonies with a drop of Kovacs Reagent. If the reagent turns a cherry-red colour in a few seconds this confirms the production of indol and hence the presence of *E. coli*. When the Chromogenic Agar for Coliform is used with the membrane filter method, the colour and growth of the colonies can be modified by the characteristics of the membrane filter. It is advisable to perform validation of the membrane filter type used.The Spanish Health Ministry (Ministerio de Sanidad y Consumo) has officially adopted this medium as an alternative methodology for the microbiological analysis of water for human consumption, giving a new definition for *Escherichia coli* ("Enterobacteriaceae that express the ß-D- galactosidase and the ß-D-glucuronidase enzymes simultaneously") and coliform bacteria: "Enterobacteriaceae that express the ß-D-galactosidase enzyme".

Technique:

The water sample is filtered trough a membrane filter of 0,45 µm of pore diameter validated according to the ISO Standard 7704:1985. The membrane is then placed on the surface of the CCA medium avoiding entrapment of air bubbles between the membrane and agar surface. The petri dish with the membrane is incubated for 18-24 hours at 36 ± 2ºC. If in 18 h there is growth of red or colourless colonies, extend the incubation until 24 h to include late reactions of ß-galactosidase or ß-glucuronidase. Count ß-galactosidase positive colonies and ß-glucuronidase negative colonies (all colonies coloured from salmon-rose to red) as Coliform bacteria not-*E. coli*. Count ß-galactosidase positive colonies and ß-glucuronidase positive colonies (all colonies coloured from deep blue to violet) as *E. coli*. Total Coliform count is obtained by the addition of the salmon-rose to red colonies plus the deep blue to violet colonies.

Reference: 101274ZA **Technical Data Sheet**

Product: **Chromocult® Coliform Agar (ISO)**

Quality control

Physical/Chemical control

Color : Pale yellow pH: 6.8 ± 0.2 at 25ºC

Microbiological control

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

Microbiological control according to ISO 11133:2014/A1:2018.

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

Aerobiosis. Incubation at 36 ± 2 ºC, reading at 18-24 h

Microorganism	Growth
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥70%) Dark-blue to violet colonies
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥70%) Dark-blue to violet colonies
Citrobacter freundii ATCC® 43864, WDCM 00006	Good (≥70%) Salmon to red colonies
Ps. aeruginosa ATCC® 10145, WDCM 00024	Good - Colourless colonies
Enterococcus faecalis ATCC® 19433, WDCM 00009	Partial Inhibition

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· ADAMS, M.., R.GRUBB, S.M. HAMER & A. CLIFFORD (1990) Colorimetric enumeration of *Escherichia coli* based on ß-glucuronidase activity. Appl. Environ. Microbiol. 56:2021.

· ISO 7704 Standard (1985) Water Quality - Evaluation of membrane filters used for micropbiological analyses.

· ISO 9308-1: 2014/Amd.1:2016(E) Water quality. Enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.

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

· KILIAN, M. & P. BÜLOW (1976) Rapid Diagnostic of Enterobacteriaceae. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B 84:245-251.

· MANAFI, M & W. KNEIFEL (1989) A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliform and *E. coli* in water. Zentralbl. Hyg. 189:225-234.

· MINISTERIO DE SANIDAD Y CONSUMO (2009) Orden SCO/778/2009 de 17 de marzo sobre métodos alternativos para el análisis microbiológico del agua de consumo humano. BOE. n.º 78 de 31-04-2009. Sección I, Págs. 30417-30420. Madrid.

Note: *Chromocult ® is a trademark of Merck KGaA. This ready to use medium is made using only Chromocult ® dehydrated media produced by Merck KGaA

Storage

Storage conditions: 2-14ºC
Alternatively the plates may also be stored at the range of 2 - 25ºC, with a proper performance of the medium, but some precautions must be taken into account:
-In the range of 2 - 8 ºC avoid direct contact with surfaces that can freeze product.
-In the range of 15 - 25 ºC, dehydration control must be taking in account.