Technical Data Sheet

Chromocult®

Coliform Agar ES (Enhanced Selectivity)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 041002

Ordering number: 1.00850.0500

For the simultaneous detection of coliform bacteria and *E. coli* in food and animal feeds. This chromogenic culture medium is selective and differential and enables the detection and enumeration of E. coli and coliforms in fresh foods within 24 hours.

Chromocult® Coliform Agar ES has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ program for the analysis of raw ground beef, raw ground chicken, and raw milk.

Mode of Action

The high level of accompanying flora in fresh food requires higher selectivity of the culture medium to ensure inhibition of unwanted bacteria and allow the target organisms to grow well. The combination of suitable peptones and the buffering using MOPS allow rapid growth of coliforms and an optimal transformation of the chromogenic substrates. The amount of bile salts and propionate largely inhibit growth of Gram-positive and Gram-negative accompanying flora.
The simultaneous detection of total coliforms and *E. coli* is achieved using the combination of two chromogenic substrates. The substrate Salmon™-β-D-GAL is split by β-D-galactosidase, characteristic for coliforms, resulting in a salmon to red colouration of coliform colonies. The detection of the β-D-glucuronidase, characteristic for *E. coli*, is cleaved via the substrate X- β-D-glucuronide, causing a dark blue colouration of positive colonies.
As *E. coli* splits Salmon™-β-D-GAL as well as X- β-D-glucuronide, the colonies turn to a dark violet colour and can be easily differentiated from the other coliforms being salmon-red.
Some *E. coli* (3-4%) are β-D-glucuronidase-negative and appear as pink to red colonies, e.g. most *E. coli* O157 strains. For the detection of *E. coli* O157 specific culture media should be used. Accompanying flora appears as colorless colonies, except for some organisms, which possess β-D-glucuronidase activity. These colonies appear light blue to turquoise in color.

For the detection and enumeration of enterococci (fecal streptococci) in water, foodstuffs and other materials according to KENNER, CLARK and KABLER (1960, 1961). KF (Kenner Fecal) Streptococcus agar complies with the recommendations given by APHA for the examination of water (1998) and foodstuffs (1992).

Typical Composition (g/L)

100850 Chromocult® Coliform Agar ES	
Peptone	5.0
Potassium chloride	7.5
MOPS	10.0
Propionate	0.5
Bile salts	1.15
6-Chloro-3-indoxyl-beta-D-galactopyranoside	0.15
5-Bromo-4-chloro-3-indoxyl-D-glucuronic acid	0.1
Isopropyl-beta-D-thiogalactopyranoside	0.1
Agar-agar*	10
Water	n.a.

* Agar-agar is equivalent to other different terms of agar.

Preparation

Dissolve 34.5 g in 1 litre of purified water. Heat in boiling water and agitate frequently until completely dissolved.

Do not autoclave, do not overheat.

Cool the medium to 45 - 50 °C in a water bath and pour plates.

For using by poured plate method cool the medium to 45 - 50 °C in a water bath before use. Do not keep the medium for longer than 2 hours in the water bath to avoid precipitation.

The prepared medium is clear and colourless.

pH: 7.2 ± 0.2 at 25 °C.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Prepared plates can be stored in sealed plastic pouches or bags for up to 2 weeks at 2 – 8 °C and protected from light.

Experimental Procedure and Evaluation

Prepare test samples using standard laboratory techniques such as those described in the applicable ISO standard, Bacteriological Analytical Manual, or other standards.

To minimize possible interference between the coloration of coliforms / *E. coli* and the sample (e.g. low pH), it is advisable to dilute the sample 1:10 in a buffered solution (e.g. add 450 ml Butterfield's Phosphate Buffer, Buffered Peptone Water, or Buffered Sodium Chloride Peptone broth to blender jar

containing 50 g of sample and mix for 2 minutes); perform further dilutions as needed.

For food analysis, Chromocult® Coliform Agar ES is usually inoculated by the pour plate method.

- Using a sterile pipette, transfer 1 ml of liquid test sample or 1 ml from the appropriate dilution to a sterile Petri dish.
- Pour into about 15 ml of the CCA at 45 - 50 °C into each Petri dish.
- Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface.
- Incubate the inoculated dishes aerobically at 35 - 37 °C in an inverted position for 24 hours.
- After incubation, examine the agar plates and count all colonies giving a positive β-D-galactosidase and β-D-glucuronidase reaction (dark-blue to violet) as *E. coli*.
- Count all colonies giving a positive β-D-galactosidase reaction (pink to red) as presumptive coliform bacteria that are not *E. coli.*

For processed food samples with a lower microbial load, e.g. processed food such as cooked chicken, non-fat dried milk and Frankfurter sausage, 110426 Chromocult® Coliform Agar acc. ISO 9308-1 (*AOAC-RI License Number 020902)* is recommended.

Validation studies – Food testing

AOAC-RI (License Number 041002). Chromocult® Coliform Agar ES has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ program for the analysis of raw ground beef, raw ground chicken, and raw milk.

The most probable number (MPN) method for coliform bacteria and E. coli (AOAC™ official method 966.24) was used for method comparison testing.

The Chromocult® Coliform ES Agar method was found to be equivalent to the AOAC™ official method.

Storage

Store the dehydrated medium dry and tightly closed. Protect from light. Do not use clumped or discolored medium. Store at +15 °C to +25 °C and use before the expiry date on the label.

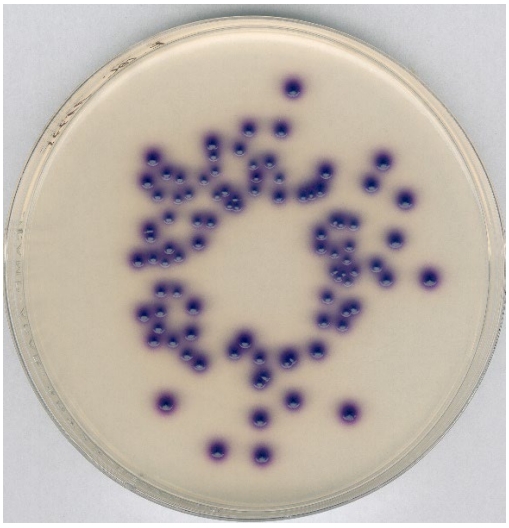
Prepared plates can be stored in sealed plastic pouches or bags for up to 2 weeks at 2 – 8 °C and protected from light.

Quality Control

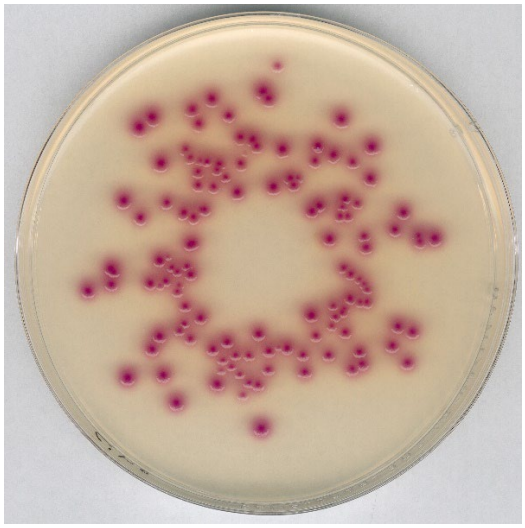
Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Escherichia coli* ATCC 11775 (WDCM 00090)	24 h at 35 °C	Tryptic Soy Agar	Quantitative	Recovery ≥ 70% dark blue colonies
	Enterobacter aerogenes ATCC 13048	24 h at 35 °C	Tryptic Soy Agar	Quantitative	Recovery ≥ 70%, pink to red colonies
	Citrobacter freundii ATCC 8090				
	Proteus mirabilis ATCC 29906 (WDCM 00023)	24 h at 35 °C	Tryptic Soy Agar	Quantitative	No recovery limit specified
Selectivity	*Enterococcus faecalis* ATCC 19433 (WDCM 00009)	24 h at 35 °C	Tryptic Soy Agar	Qualitative	inhibition
	Aeromonas hydrophila ATCC 7966 (WDCM 00023)	24 h at 35 °C	Tryptic Soy Agar	Qualitative	inhibition
	Staphylococcus aureus ATCC 25923 (WDCM 00034)	24 h at 35 °C	Tryptic Soy Agar	Qualitative	inhibition
	Bacillus subtilis ATCC 6633 (WDCM 00003)	24 h at 35 °C	Tryptic Soy Agar	Qualitative	inhibition

Please refer to the actual batch related Certificate of Analysis.

A recovery rate of 70 % is equivalent to a productivity value of 0.7.



Escherichia coli ATCC 11775



Citrobacter freundii ATCC 8090

Literature

Frampton, E.W., Restaino, L., & Blaszko, L. (1988) *J. Food Prot.* **51**, 402 – 404

Kilian, M., & Bülow, P. (1976) Rapid diagnosis of Enterobacteriaceae. I. Detection of bacterial glycosidases. *Acta Pathol. Microbiol. Scand. Sect. B.* **84**, 245 – 251 5

Manafi, M., & Kneifel, W.A. (1989) *Zentralbl. Hyg.* **189**, 225 – 234 American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3 rd. ed., 1992.

Ordering Information

Product	Cat. No.	Pack size
Chromocult® Coliform Agar ES	1.00850.0500	500 g
Chromocult® Coliform Agar acc. ISO 9308-1	1.10426.0500	500 g