Technical Data Sheet

Chromocult® Coliform Agar acc. ISO 9308-1

Ordering number: 1.10426.0500

For the simultaneous detection of coliform bacteria and E. coli in drinking water, waters with low bacterial background flora and processed food samples.

Drinking water and waters with low bacterial background: This culture medium complies with the specifications given by EN ISO 9308-1.

Chromocult® Coliform Agar membrane filter method has been approved and accepted by the U.S. Environmental Protection Agency (EPA) for monitoring total coliform bacteria and *E. coli* under the Total Coliform Rule (40 CFR 141.21).

Processed food: Chromocult® Coliform Agar acc. ISO 9308-1 (CCA) has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ program for the analysis of frankfurters, cooked chicken and non-fat dried milk.

Mode of Action

The interaction of selected peptones, pyruvate, sorbitol and phosphate buffer promotes rapid colony growth, even for sub-lethally injured coliform bacteria. The growth of Gram-positive bacteria as well as certain Gram-negative bacteria is inhibited by the presence of Tergitol®7 which has no negative effect on the growth of coliform bacteria.

Further, the combination of two chromogenic substrates permits the simultaneous detection of coliform bacteria and *E. coli.*

Coliform bacteria detection: The substrate Salmon-GAL is used for the detection of β-D-galactosidase activity, which is characteristic for coliform bacteria. This interaction results in a pink to red color of the coliform colonies.

***E. oli* detection:** The substrate X-glucuronide is used for the detection of β-D-glucuronidase activity, which is characteristic for *E. coli.*

E. coli cleaves both Salmon-GAL and X-glucuronide, so that positive colonies take on a dark-blue to violet color. These are easily distinguished from other coliform colonies, which have a pink to red color.

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.

Typical Composition

Specified by ISO 9308-1		Chromocult® Coliform Agar	
Enzymatic Digest of Casein	1 g/l	Enzymatic Digest of Casein	1 g/l
Yeast Extract	2 g/l	Yeast Extract	2 g/l
NaCl	5 g/l	NaCl	5 g/l
NaH_2PO_4 x 2 H_2O	2.2 g/l	NaH_2PO_4 x 2 H_2O	2.2 g/l
Na_2HPO_4	2.7 g/l	Na_2HPO_4	2.7 g/l
Sodium Pyruvate	1 g/l	Sodium Pyruvate	1 g/l
Sorbitol	1 g/l	Sorbitol	1 g/l
Tryptophane	1 g/l	Tryptophane	1 g/l
Secondary alcohol ethyloxylate surfactant (CAS No. 68131-40-8) (e.g. Tergitol® 15-S-7 surfactant)	0.15 g/l	Tergitol® 7	0.15 g/l
6-chloro-3-indoxyl-beta-D-galactopyranoside (Salmon-beta-D-galactosid), (CAS No. 138182-21-5)	0.2 g/l	6-Chloro-3-indoxyl-beta-D-galactopyranoside	0.2 g/l
5-Bromo-4-chloro-3-indoxyl-β-D-glucuronic acid, cyclohexylammonium salt monohydrate (X-beta-D-glucuronide CHX salt), (CAS No. 114162-64-0)	0.1 g/l	5-Bromo-4-chloro-3-indoxyl-D-glucuronic acid	0.1 g/l
Isopropyl-β-D-thiogalactopyranoside (IPTG), (CAS No. 367-93-1)	0.1 g/l	Isopropyl-beta-D-thiogalactopyranoside	0.1 g/l
Bacteriological agar	9-18 g/l	Agar-agar*	10 g/l
Water	1000 ml	Water	n.a.

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

Preparation

Dissolve 26.5 g in 1 liter 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 and pour plates. According to EN ISO 9308-1 dispense in Petri dishes to a depth of at least 4 mm (approximately 18 ml in a 90 mm plate).

For using by poured plate method cool the medium to 44 - 47 °C in a water bath before use (see EN ISO 11133).

The prepared medium is slightly turbid to turbid and yellowish. The pH value at 25 °C is in the range of 6.6-7.0

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.

Preparation and addition of optional Cefsulodin solution (US-EPA approved method): Dissolve 5 mg of Cefsulodin in 2 ml of purified water and sterilize by membrane filtration (0.2 µm nominal pore size). Aseptically add the solution (2 ml per 500 ml of medium) to 500 ml of liquefied medium (45 - 50 °C). Pour plates as described above.

Experimental Procedure and Evaluation

Prepare test samples using standard laboratory techniques such as those described in the applicable ISO standard, Bacteriological Analytical Manual, or Standard Methods for the Examination of Water and Wastewater specific for the product concerned.

Drinking water and waters with low bacterial background: Chromocult® Coliform Agar acc. ISO 9308-1 is usually combined with membrane filtration for water analysis.

Note: The type and quality of membrane filter affects the size, coloration and number of colonies significantly. Merck Millipore mixed cellulose ester filters (gridded, 0.45 µm pore size, EZ-PakTM, Merck, article number EZHAWG474) were used in ISO validation studies (Lange et al. 2013) and supported the color formation and the growth of colonies efficiently.

- Filter appropriate volume of sample (e.g. 100 ml municipal drinking water, 250 ml bottled water) using a membrane filter.
- Place filter on CCA plate ensuring that no air is trapped underneath.
- Incubate the inoculated dishes aerobically in an inverted position at 36 °C ± 2°C for 21-24 h

Note: Do not incubate longer than 24 h to reduce risk of counting unwanted microorganisms with similar colony colors.

- After incubation, examine the membrane filters 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.*
- Confirm pink to red colonies by a negative oxidase reaction (to avoid false-positive results caused by oxidase-positive bacteria like *Aeromonas* spp.).

Note: In ISO validation studies oxidase activity of presumptive coliform colonies was tested using Bactident® Oxidase (article number 113300) test.

Processed food

For food analysis, Chromocult® Coliform Agar acc. to ISO 9308-1 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 44 - 47 °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 plates for presence of typical colored colonies of E. coli and other coliform bacteria.

For fresh food samples with a higher microbial load, Chromocult® Coliform Agar ES (article number 100850) is recommended.

Validation studies - Water testing

EN ISO 9308-1:2014. The performance of Chromocult® Coliform Agar was validated according to ENV ISO 13843 ("Water quality – Guidance on validation of microbiological methods") using pure cultures and naturally contaminated water samples. Samples were processed according to ISO 9308-1 using EZ-Pak membrane filters (Merck) for filtration. In total 220 colonies, including typical *E. coli* and coliform bacteria as well as atypical colonies, were randomly selected to determine the fundamental characteristics of CCA.

The results show that Chromocult® Coliform Agar acc. ISO 9308-1 is a reliable method for the quantification of both *E. coli* and coliform bacteria.

US-EPA. Chromocult® Coliform Agar membrane filter method has been approved and accepted by the U.S. Environmental Protection Agency (EPA) for monitoring total coliform bacteria and *E. coli* under the Total Coliform Rule (40 CFR 141.21).

In validation studies for the detection of total coliform bacteria and *E. coli* in drinking water the Chromocult® Coliform Agar supplemented with 2,5 mg / L Cefsulodin method was compared to the EPA approved reference method (mENDO LES Agar).

The Chromocult® Coliform Agar method offered precise and accurate identification and measurement of total coliform bacteria and *E. coli.*

Food testing

AOAC-RI (License Number 020902). Chromocult® Coliform Agar has been validated by the AOAC™ Research Institute under the Performance Tested Methods℠ program for the analysis of frankfurters, cooked chicken and non-fat dried 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 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 dis-colored medium. Store at +15 °C to +25 °C and use before the expiry date on the label.

According to EN ISO 9308-1, self-prepared plates can be stored at +2 °C to +8 °C in the dark and protected against evaporation for at least one month.

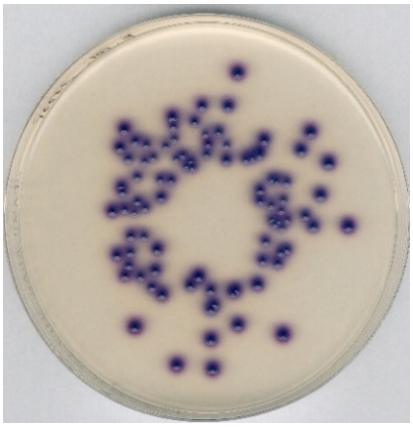
Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Escherichia coli* ATCC 25922	18-24 h at 35-37 °C	Tryptic Soy Agar	Quantitative with membrane filtration	Recovery ≥ 70 %, dark-blue to violet colonies
	Escherichia coli ATCC 8739				
	Enterobacter aerogenes ATCC 13048	18-24 h at 35-37 °C	Tryptic Soy Agar	Quantitative with membrane filtration	Recovery ≥ 70 %, pink to red colonies
	Citrobacter freundii ATCC 43864				
Selectivity	*Enterococcus faecalis* ATCC 19433	18-24 h at 35-37 °C		Qualitative with membrane filtration quantitative	Partial inhibition, colorless colonies
Specificity	*Pseudomonas aeruginosa* ATCC 10145	18-24 h at 35-37 °C		Qualitative with membrane filtration quantitative	No recovery limit specified, colorless colonies

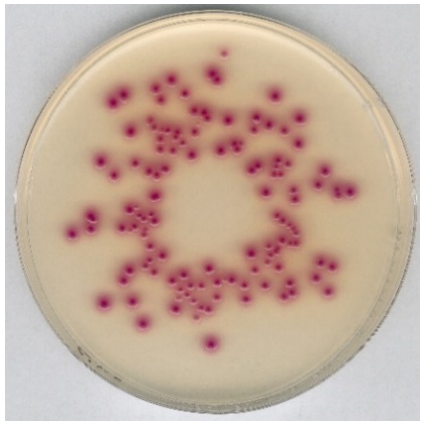
Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133 and EN ISO 9308-1 using Merck Millipore mixed cellulose ester filters (0.45 µm pore size).

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



E. coli ATCC 11775



Citrobacter freundii ATCC 8090

Literature

ISO International Standardisation Organisation. Water quality – Enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora. EN ISO 9308-1:2014.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

US-EPA approved method. 40 CFR Part 141 (sec 141.21) Federal Register/Vol. 67, No. 209, Tuesday October 29, 2002/Rules and Regulations.

New Zealand Dairy Industry. (1998): Microbiological Methods Manual, Section 48: Product Test Methods - Enteric Indicator Organisms. NZTM 2: 48.5.1-48.5.10.

Byamukama, D., Kansiime, F., March R.L. and Farnleitner A.H. (2000): Determination of *Escherichia coli* contamination with Chromocult Coliform Agar showed a high level of discrimination efficiency for differening fecal pollution levels in tropical waters of Kampala, Uganda. Appl. Environ. Microbiol. 66: 864-868.

Geissler, K., Manafi, M., Amoros, I. and Alonso J.L. (2000): Quantitative determination of total coliforms and *Escherichia coli* in marine waters with chromogenic and fluorogenic media. J. Appl. Microbiol. 88: 280-285.

Lange, B., Strathmann, M. and Oßmer, R (2013): Performance validation of chromogenic coliform agar for the enumeration of *Escherichia coli* and coliform bacteria. Lett. Appl. Microbiol. 57: 547-553.

Ordering Information

Product	Cat. No.	Pack size
Chromocult® Coliform Agar acc. ISO 9308-1	1.1.0426.0500	500 g
ReadyPlate™ CHROM Chromocult® Coliform Agar acc. ISO 9308-1:2014	1.46689.0020	20 x 90 mm plates
ReadyPlate™55 Chromocult® Coliform Agar acc. ISO 9308-1:2014	1.46757.0020	20 x 55 mm plates
ReadyPlate™55 Chromocult® Coliform Agar acc. ISO 9308-1:2014	1.46757.0200	200 x 55 mm plates
Chromocult® Coliform Agar ES	1.00850.0500	500 g
EZ-Pak™ cellulose mixed ester filter (gridded, 0,45 µm pore size)	EZHAWG474	4 bands x 150 membrane filters
S-Pak™ cellulose mixed ester filter (gridded, 0,45 µm pore size)	HAWG047S6	4 boxes of 600 membrane filters, individually packed
Bactident® Oxidase	1.13300.0001	50 strips

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