

Technical Data Sheet

ReadyPlate™55 KIT Chromocult® Coliform Agar acc ISO 9308-1:2014 Ordering number: 1.46758.0150

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

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

General

This Kit contains Chromocult® Coliform Agar that complies with the specifications given by EN ISO 9308-1:2014 and EZ Pak Membrane Filter according to ISO 7704. It is released through an ISO 17025 accredited lab in compliance of the specifications stated in ISO 11133.

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).



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. coli* 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 | | ReadyPlate™ 55 Chromocult® | |
|--|----------|---|-----------|
| 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 ₂ PO ₄ x 2 H ₂ O | 2.2 g/l | NaH ₂ PO ₄ x 2 H ₂ O | 2.2 g/l |
| Na ₂ HPO ₄ | 2.7 g/l | Na ₂ HPO 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 | 1000 ml/l |

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

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

Protect plates from prolonged exposure to light during preparation and application, as this may affect the coloration of the colonies.

Application and Interpretation

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

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. MilliporeSigma mixed cellulose ester filters (gridded, 0.45 µm pore size, EZ-Pak™, 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 $36\text{ °C} \pm 2\text{ °C}$ 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 test.

Note: Protect plates from prolonged exposure to light during preparation and application, as this may affect the coloration of the colonies

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 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.

Storage and Shelf Life

The product can be used for tests until the expiry date if stored upright, protected from light and properly sealed at $+15\text{ °C}$ to $+25\text{ °C}$.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C , disinfect, incinerate etc.).

Quality Control

| Function | Control strains | Incubation | Reference medium | Method of control | Expected results |
|--------------|--|---------------------|------------------------|---|---|
| Productivity | <i>Escherichia coli</i> ATCC 25922 | 18-24 h at 35-37 °C | Tryptic Soy Agar (TSA) | Quantitative with membrane filtration | Recovery ≥ 70 %, dark-blue to violet colonies |
| | <i>Escherichia coli</i> ATCC 8739 | | | | |
| | <i>Enterobacter aerogenes</i> ATCC 13048 | | | Quantitative with membrane filtration | Recovery ≥ 70 %, pink to red colonies |
| | <i>Citrobacter freundii</i> ATCC 43864 | | | | |
| Specificity | <i>Pseudomonas aeruginosa</i> ATCC 10145 | 18-24 h at 35-37 °C | - | Qualitative with membrane filtration quantitative | No recovery limit specified, colorless colonies |
| Selectivity | <i>Enterococcus faecalis</i> ATCC 19433 <i>Enterococcus faecalis</i> ATCC 29212 | 18-24 h at 35-37 °C | - | Direct Inoculation | Total or Partial inhibition |

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 MilliporeSigma mixed cellulose ester filters (0.45 µm pore size).

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

Literature

Byamukama, D., Kansime, 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 differencing 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.

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.

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.

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

Ordering Information

| Product | Cat. No. | Pack size |
|--|-----------------|--------------------------------|
| ReadyPlate™55 KIT CCA ISO 9308 | 1.46758.0150 | KIT* |
| ReadyPlate™55 CCA ISO 9308 | 1.46757.0020 | 20 x 55mm plates |
| ReadyPlate™55 CCA ISO 9308 | 1.46757.0200 | 200 x 55mm plates |
| ReadyPlate™ CHROM CCA ISO 9308 | 1.46689.0020 | 20 x 90mm plates |
| Chromocult® Coliform Agar acc. ISO 9308-1 | 1.10426.0500 | 500 g |
| EZ-Pak® Membrane Dispenser Curve | EZCURVE01 | 1 unit |
| EZ-Pak™ cellulose mixed ester filter (gridded, 0,45 µm pore size) | EZHAWG474 | 4 bands x 150 membrane filters |
| Bactident® Oxidase | 1.13300.0001 | 50 strips |

* The KIT Contains 150 x 55mm Media and 1 box x150 membrane Filters.

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The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

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