

TECHNICAL DATA SHEET

CHROMOGENIC COLIFORM AGAR (CCA)

ENUMERATION OF *ESCHERICHIA COLI* AND COLIFORMS

1 INTENDED USE

Chromogenic Coliform Agar (CCA) is used for the detection and enumeration of *Escherichia coli* and coliform bacteria in waters of low bacterial numbers (less than 100 total colonies). These waters can be drinking water, disinfected pool water or finished water from treatment plants.

The typical composition complies with the formula described in the standard ISO 9308-1: 2014.

2 PRINCIPLES

The simultaneous presence of two substrates enables the detection of the two specific enzyme activities: β -galactosidase and β -glucuronidase.

Coliforms are distinguished by the production of β -galactosidase (β -gal). This enzyme reacts with the chromogenic substrate to produce a pink to red precipitate.

All *Escherichia coli* possess β -galactosidase and 94 - 97 % are also positive for β -glucuronidase (GUD). The presence of the enzyme is revealed by the presence of a blue coloration. The simultaneous action of the two enzymes gives the colonies of *Escherichia coli* a purple color.

3 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal results.

For 1 liter of media:

- Enzymatic digest of casein.....	1.0 g
- Yeast extract	2.0 g
- Sodium chloride	5.0 g
- Sodium dihydrogen phosphate x 2H ₂ O.....	2.2 g
- Di-sodium hydrogen phosphate	2.7 g
- Sodium pyruvate	1.0 g
- Sorbitol	1.0 g
- Tryptophan	1.0 g
- Secondary alcohol ethyloxylate surfactant.....	0.15 g
- 6-Chloro-3-indoxyl- β -D-galactopyranoside	0.2 g
- 5-Bromo-4-chloro-3-indoxyl- β -D-glucuronic acid	0.1 g
- Isopropyl- β -D-thiogalactopyranoside (IPTG)	0.1 g
- Bacteriological agar.....	16.0 g

pH of the ready-to-use media at 25 °C: 6.8 \pm 0.2.

4 PREPARATION

- Dissolve 32.5 g of dehydrated media (BK204) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Do not autoclave.
- Cool the medium to 44-47°C.
- Distribute in sterile Petri dishes (in order to obtain a thickness of at least 5 mm).
- Let solidify on a cool surface.

✓ **Reconstitution:**
32.5 g/L

✓ **Sterilization**
Bring to boil only

Note: Shield the medium from light.

5 INSTRUCTIONS FOR USE

- Aseptically filter through a membrane the volume of water to test.
- Deposit the membrane on the surface of the prepared plates or the pre-poured agar (BM182), filtered side up and taking care to keep the membrane and the agar in close contact. The plates should be brought to room temperature before use.
- Incubate at $(36 \pm 2) ^\circ\text{C}$ for 21 to 24 hours.

✓ **Inoculation:**
Membrane filtration

✓ **Incubation:**
21 to 24 h at 36°C

6 RESULTS

Enumerate separately the *E. coli* and coliforms according to the following table:

Microorganisms	Typical phenotype	Color of colonies
<i>Escherichia coli</i>	GUD + / β -gal +	Dark blue to violet
Coliforms other than <i>E. coli</i>	GUD - / β -gal +	Pink to red

To ease the reading, results can be read by direct count through the bottom of the plates.

In case of doubt as to the pink coloration of coliforms (β -galactosidase activity vs. natural pigmentation of the strain), it is advised to slightly raise the filter. The coloration linked to the chromogenic activity is visible on the agar.

See ANNEX 1: PHOTO SUPPORT.

According to the standard ISO 9308-1, coliforms other than *E. coli* should be confirmed with a negative reaction to the oxidase test.

7 QUALITY CONTROL

Dehydrated media: cream powder, free-flowing and homogeneous.

Prepared media: amber agar.

Typical culture response after 21 hours of incubation at 36°C (ISO 9308-1/A1):

Microorganisms	WDCM	Growth (Productivity Ratio: P_R)
<i>Escherichia coli</i>	WDCM 00012	$P_R \geq 70 \%$, blue to violet colonies
<i>Escherichia coli</i>	WDCM 00013	$P_R \geq 70 \%$, blue to violet colonies
<i>Citrobacter freundii</i>	WDCM 00006	$P_R \geq 70 \%$, pink colonies
<i>Enterobacter aerogenes</i>	WDCM 00175	$P_R \geq 70 \%$, pink colonies
<i>Pseudomonas aeruginosa</i>	WDCM 00024	Inhibited or no characteristics colonies
<i>Enterococcus faecalis</i>	WDCM 00087	Inhibited

8 STORAGE / SHELF LIFE

Dehydrated media: $2-30 ^\circ\text{C}$.

Pre-poured media: $2-8 ^\circ\text{C}$, shielded from light.

The expiry dates are indicated on the labels.

Prepared media in Petri dishes (*): 30 days at $2-8 ^\circ\text{C}$, shielded from light.

(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

9 PACKAGING

Pre-poured plates:

20 plates \varnothing 55 mm..... BM18208

Dehydrated media:

500 g bottle BK204HA

10 BIBLIOGRAPHY

B. Lange, M. Strathmann and R. Oßmer. Performance validation of chromogenic coliform agar for the enumeration of *Escherichia coli* and coliform bacteria. Letters in Applied Microbiology Volume 57, Issue 6, pages 547–553, December 2013.

ISO 9308-1. September 2014. Water quality - Enumeration of *Escherichia coli* and coliform bacteria - Part 1: membrane filtration method for waters with low bacterial background flora

ISO 9308-1/A1. December 2016. Water quality — Enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora. AMENDMENT 1

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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Chromogenic Coliform agar (CCA)

Enumeration of *Escherichia coli* and other coliform bacteria in water.

Results :

Growth obtained after 21 hours of incubation at 36 °C.

