



User manual

Chromogenic Coliform Agar (Product No. 8.43330.244)

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1. Information

Chromogenic Coliform Agar (CCA) is used for the quantitative detection and differentiation of *Escherichia coli* and coliform bacteria in water samples.

Chromogenic Coliform Agar (pH 6.8 ± 0.2) is a ready-to-use, selective and differential chromogenic agar used for the detection and enumeration of *Escherichia coli* and coliform bacteria in water samples with low bacterial background flora, according to ISO 9308-1 and the new German Drinking Water Ordinance (Trinkwasserverordnung).

The simultaneous detection of Coliforms and *E. coli* is made possible by the combination of two chromogenic substrates. The chromogenic substrate Salmon[®]-GAL (6-chloro-3-indolyl-β-D-galactopyranoside) is cleaved by the β-D-galactosidase characteristic of Coliforms and colours positive colonies pinkish-red.

The cleavage of the chromogenic substrate X-glucuronide (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) by the β-D-glucuronidase characteristic of *E. coli* colours the positive colonies blue. Because *E. coli* cleaves both Salmon[®]-GAL and X-glucuronide, the colour of the colonies is dark blue to violet. These colonies can be easily distinguished from the other Coliforms, which are pinkish-red. Tryptophan in the medium improves the indole reaction for the further confirmation of *E. coli* and improves the detection reliability.

2. Handling

Required Material

Laminar flow workbench
Water bath
Petri dishes
Incubator
Membrane filtration system and filter membrane (0.45 µm pore diameter) or
Drigalski spatula/sterile inoculation loop

Application

Please work under sterile conditions to avoid secondary contamination of the samples.

Melt the bottle content in a water bath at 95°C/203°F. Avoid overheating and remove the medium as soon as it is completely dissolved. The screw cap should be loosened before heating and close tightly after removing from the water bath. To avoid glass breakage, it is recommended to leave the bottles for a short period, e.g. 2 min, on a head-resistant surface at room temperature. Subsequently place them in a 45-50°C/113-122°F water bath for cooling. Please ensure that the agar is not solidified again.

Do not leave the liquefied agar for longer than 4 h at 45-50°C/113-122°F in a water bath. Repeated heating can restrain its functionality.

ISO 9308-1 recommends filtering the water samples through a filter membrane (0.45 µm pore diameter). Then transfer the membrane to a petri dish with CCA Agar.



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Alternatively, samples can be inoculated by pouring, plating or with a sterile inoculation loop and swabbed directly onto the medium.

Incubation

Incubate the samples aerobically at 34-38°C/93-100°F for 18-24 hours. Incubation conditions may vary depending on the microorganisms to be studied.

Evaluation

Examine the colour of the colonies and interpret the results as indicated in the identification table below. Document the colony number per gram or per mL of sample.

Table 1: Identification table

Microorganisms	Typical colony colour
β -D-galactosidase and β -glucuronidase-positive <i>E. coli</i> *	Dark blue to violet
Other coliform bacteria**	Pink to red
Other bacteria – if not inhibited	Colourless

* β -glucuronidase-negative *E. coli* strains, such as *E. coli* O157, are pink to red on this medium.

Some strains of *Shigella* and *Salmonella* that produce the enzyme β -glucuronidase can grow as light blue colonies.

**Perform an oxidase test to confirm coliform bacteria that are oxidase negative.

3. Storage and Packaging Information

Packaging and Content

unit Cardboard Box (6x200 mL in glass bottles)
unit size (Box) approx. 22 cm x 14 cm x 16 cm / 8.7 in x 5.5 in x 6.3 in
unit gross weight (Box) approx. 2.2 kg/ 4,9 lbs.

Storage

Store at 2-8°C/50-77°F according to product specification.
Store under dry and dark conditions.

Waste Disposal

No dangerous good.

No hazardous material.

Please consider your local waste regulations.

Not inoculated media can be disposed of with normal laboratory waste.

Inoculated and incubated media are to be sterilized before disposal at a temperature of media 121°C /250°F for 20 min.



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Warnings

Do not boil or freeze product.
Always wear protective clothing when handling hot media.

4. Related Products

Target micro-organisms	Sample type	Product	Format	Item no.	Packaging	pH	Incubation		
							T [°C]	t [d]	Condition
Total viable micro-organism count (TVC)	General, Drinking, table & mineral water, Rinsing water, Beer & beer mix beverages, Wine & wine mix beverages, Soft drinks (<30% juice), Juice & nectars, Dairy & milk mix beverages	PCA	Agar	8.43221.244	6 x 200 ml (glass bottles)	7.0 ± 0.2	30 ± 1	3	aerobic
		TSB	Broth	8.43246.244	6 x 200 ml (glass bottles)	7,3 ± 0,2	30 - 35 20 - 25	< 3 5	aerobic
		TSA	Agar	8.43326.244	6 x 200 ml (glass bottles)	7.3 ± 0.2	30 ± 1	2	aerobic
	Drinking, table & mineral water, rinsing water	Nutrient Agar DEV	Agar	2.04726.782	9 x 250 ml (glass bottles)	7.2 ± 0.2	20 or. 37 ± 2	1 - 3	aerobic
E. coli & Coliforms	Drinking, table & mineral water, rinsing water	LMC	Concentrated Broth	2.04713.700	9 x 50 ml (glass bottles)	7.2 ± 0.2	37 ± 2	2	aerobic
		VRBL	Agar	8.43331.244	6 x 200 ml (glass bottles)	7.4 ± 0.2	30 or 37	1	aerobic