

COLIFORM CHROMOGENIC AGAR (CCA)

(ISO 9308-1)

 Chromogenic medium for detection and enumeration of *E. coli* and coliform bacteria in water, according to ISO 9308-1.

DESCRIPTION

Coliform Chromogenic Agar (CCA) is a selective and differential chromogenic medium 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.



PRINCIPLE

Enzymatic digest of casein provides amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Yeast extract is a source of vitamins, particularly of B-group. Sodium chloride maintains the osmotic balance of the medium. Phosphates act as buffer. Sodium pyruvate enhances recovery of injured organisms. Sorbitol is the fermentable carbohydrate. Tryptophan is incorporated into the medium to make possible performing indole test for confirmation of *E. coli*. Tergitol® 7 (sodium heptadecylsulfate) is a surfactant that inhibits Gram-positive bacteria. Salmon®-GAL (6-chloro-3-indolyl-β-D-galactopyranoside) is the substrate of β-D-galactosidase, an enzyme typically found in coliform bacteria. X-Glucuronide (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) is the other chromogenic substrate cleaved by the β-D-glucuronidase enzyme characteristic of *E. coli*. The combination of these two substrates allows to differentiate *E. coli* from other coliforms and Gram-negative bacteria on the basis of the color of the colonies. IPTG (isopropyl-β-D-thiogalactopyranoside) is an inducer for the expression of β-D-galactosidase. Agar is the solidifying agent.

COMPOSITION	g/L
Enzymatic digest of casein	1,00
Yeast Extract	2,00
Sodium chloride	5,00
Sodium dihydrogen phosphate	2,20
Disodium hydrogen phosphate	2,70
Sodium pyruvate	1,00
L-Tryptophan	1,00
Sorbitol	1,00
Tergitol®7	0,15
6-Chloro-3-indoxyl- β-D-galactopyranoside (Salmon-beta-D-galactosid)	0,20
5-Bromo-4chloro-3-indoxyl- β-D-glucuronic acid, cyclohexylammonium salt monohydrate (X-beta-G-glucuronide CHX salt)	0,10
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0,10
Agar	11.55

Final pH 6,8 ± 0,2 at 25°C

WARNING AND PRECAUTIONS

For in vitro diagnostic use.

Observe the precautions normally taken when handling laboratory reagents.

Dehydrated medium: HIGHLY HYGROSCOPIC. During the handling, wear dust protection mask. Avoid the eye contact. Do not use beyond the expiration date or if the product shows signs of deterioration, an altered color or has compacted.

Prepared Medium: The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous.

Safety Data Sheet is available on request for professional users.

All waste must be disposed of according to local directives.

STORAGE AND STABILITY

Dehydrated medium:	2-8°C
Prepared medium:	2-8°C

The product is stable until the expiration date indicated on the label under the recommended storage conditions.

PREPARATION

Dehydrated medium: Suspend 28.0 g of Coliform Chromogenic Agar (CCA) in 1 liter of distilled water. Slowly bring to a boil until completely dissolved. DO NOT AUTOCLAVE. Cool to approximately 50 ° C and distribute the medium in sterile petri dishes.

Prepared medium (bottles): Melt the content of the bottle in a water bath at 100°C until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

PROCEDURE

ISO 9308-1 recommends to filter the water sample through a filter membrane (0.45 μm pore diameter), transfer the membrane onto a Coliform Chromogenic Agar plate and incubate aerobically at 36 ± 2°C for 21 to 24 hours.

RESULTS

MICROORGANISM	TYPICAL COLONY COLOR
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)	Colorless

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

A few strains of *Shigella* and *Salmonella* which produce the enzyme β-glucuronidase can grow as light blue colonies.

Carry out an oxidase test to confirm coliform bacteria which are oxidase-negative.

QUALITY CONTROL

Dehydrated medium: pale pink powder, homogeneous and free of lumps.

Prepared medium: Slightly opalescent, light amber.

Typical response after incubation at 36±2°C for 21-24 hours, in aerobiosis

MICROORGANISM	GROWTH
<i>Escherichia coli</i> WDCM 00013	Dark-blue to violet colonies
<i>Enterobacter aerogenes</i> WDCM 00175	Pink to red colonies
<i>Enterococcus faecalis</i> WDCM 00009	Total or partial inhibition
<i>Pseudomonas aeruginosa</i> WDCM 00024	Colorless colonies

REFERENCES

- ISO 9308-1: 2017. Water quality – Enumeration of *Escherichia Coli* and Coliform bacteria. Part 1: Membrane filtration method for water with low bacteria background flora.

PRESENTATION

	Packaging	REF.
Terreno Disidratato COLIFORM CHROMOGENIC AGAR (CCA)	100 g (3.5 L)	11504
	500 g (18.8 L)	10504
Terreno pronto: COLIFORM CHROMOGENIC AGAR (CCA)	6 x 100 mL Bottles	63638
	12 x 200 mL Bottles	63538
	20 pcs (60 mm ready-to-use plates)	3623010/20
	20 pcs (90 mm ready to use plates)	1110203/20

SYMBOLS



Read the instructions



Biological hazard



CE Mark (product complies with the requirements of Regulation (EU) 746/2017)



Temperature limitation



Use by



For in vitro diagnostic use



Manufacturer