



## CHROMOGENIC COLIFORM AGAR ISO FORMULATION

### Cultural response



*E. coli*: blue colonies; *Citrobacter freundii*: red colonies

### Typical formulas

#### Dehydrated and ready to use media (g/L)

Enzymatic digest of casein	1,00
Yeast extract	2,00
Sodium chloride	5,00
Sodium dihydrogen phosphate x 2H <sub>2</sub> O	2,20
Di-sodium hydrogen phosphate	2,70
Sodium pyruvate	1,00
Sorbitol	1,00
Tryptophane	1,00
Tergitol® 7	0,15
Salmon-β-D-galactoside	0,20
X-β-glucuronide CHX salt	0,10
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0,10
Bacteriological agar	10,60

### Intended use

Powdered medium and ready to use plates for the enumeration of *Escherichia coli* and coliform bacteria with membrane filtration method for waters with low bacterial background, according to ISO 9308-1:2014

### Principle of the method and explanation

Chromogenic Coliform Agar ISO Formulation is a selective and differential medium for the simultaneous enumeration of *E. coli* and coliform bacteria in waters with low bacterial background with the membrane filtration technique, according to the ISO Standard 9308-1:2014.

The medium is made selective by the presence of Tergitol 7 an inhibitor of Gram positive bacteria. The differentiation between coliform bacteria, different from *E. coli* and *E. coli* is given by the presence of Salmon-β-D-galactoside, a chromogenic substrate for the detection of β-galactosidase and X-β-Glucuronide, a chromogenic substrate for the detection of β-glucuronidase.

Salmon-β-D-galactoside is hydrolysed by coliforms releasing a pink-red colour; this reaction is strengthened in the medium by the presence of IPTG (isopropil-β-D-thiogalactopiranoside).

X-β-glucuronide is hydrolysed, among *Enterobacteriaceae*, by *E. coli*, and by a few strains of *Salmonella* and *Shigella* releasing a blue pigment.

### Directions for preparation from dehydrated medium

Suspend 27,1 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation until completely dissolved. Do not autoclave, do not overheat. Some turbidity may occur after boiling; it normally disappears when the temperature decreases to 45-50 °C and doesn't affect the medium performances

Dispense in petri dishes to a depth of at least 4 mm. If not for immediate use, the plates can be stored at 5 ± 3 °C in the dark and protected against evaporation for not more than one month. 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.

### Physical characteristics

Dehydrated medium appearance: yellowish, fine, homogeneous, free-flowing powder.

Prepared medium appearance: light yellow, limpid or slightly opalescent.

Final pH: 6,8 ± 0,2



## Technique

Filter 100 ml (or other volumes, e.g. 250 ml for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 ml of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.

After filtration place the membrane filter on the Chromogenic Coliform Agar ISO Form., ensuring that no air is trapped underneath, invert petri dish, and incubate at  $36 \pm 2$  °C for 18-24 h.

Examine the membrane filters and count:

- All pink to red colonies (positive for  $\beta$ -D-galactosidase reaction) as presumptive coliform bacteria that are not *E. coli*.
- All dark-blue to violet colonies (positive for  $\beta$ -D-galactosidase and  $\beta$ -D-glucuronidase reactions) as *E. coli*.

To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp, the presumptive colonies shall be confirmed by a negative oxidase reaction (Oxidase test Strips, cat. N° 191040ST)

Perform the oxidase test preferentially on all, or at least 10 selected pink to red colonies (coliform bacteria, different from *E.coli*), the test should be negative.

The count of total coliform bacteria is the sum of all oxidase negative pink to red colonies plus all dark-blue to violet colonies.

## Limitations

Some strains of *E. coli* which are  $\beta$ -D-glucuronidase negative, such as *Escherichia coli* O157, will not be detected as *E. coli*. As they are  $\beta$ -D-galactosidase positive, they will appear as coliform bacteria on this chromogenic agar.

## Quality control: microbiological characteristics

CONTROL STRAINS	INOCULATION INOCUBATION			SPECIFICATIONS	
<i>E. coli</i>	ATCC	25922	Q 37°C /24H / A	BLUE COLONIES	A/C $\geq$ 0,7
<i>E. coli</i>	ATCC	8739	Q 37°C /24H / A	BLUE COLONIES	A/C $\geq$ 0,7
<i>E.aerogenes</i>	ATCC	13048	Q 37°C /24H / A	PINK COLONIES	A/C $\geq$ 0,7
<i>C.freundii</i>	ATCC	43864	Q 37°C /24H / A	RED COLONIES	A/C $\geq$ 0,7
<i>P.aeruginosa</i>	ATCC	10145	E 37°C /24H / A	GOOD GROWTH COLOURLESS COLONIES	
<i>E. faecalis</i>	ATCC	19433	MM 37°C /24H / A	GROWTH INHIBITED	

A: AEROBIC INCUBATION

A/C (Productivity Ratio): CFU OBTAINED ON TEST BATCH / CFU OBTAINED ON TRYPTIC SOY AGAR

Q: QUANTITATIVE MF (MEMBRANE FILTRATION) METHOD / E: ECOMETRY / MM: MODIFIED MILES MISRA

## Storage

Dehydrated medium REF 4012972: keep tightly closed, away from bright light, at +2°C to +8 °C

User prepared plates : up to 1 month at +2°C to +8 °C, in the dark, protected against evaporation.

Ready to use plates (REF 491297): keep at +2°C to +8 °C

## Precautions

The products described are for Laboratory use only.

## References

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

## Ordering information

Product	Type	Cat. N°	Pack size
CHROMOGENIC COLIFORM AGAR ISO FORMULATION	DCM	4012972	500 g (18,4 L)
CHROMOGENIC COLIFORM AGAR ISO FORMULATION	Ready to use plates Ø 55 mm	491297	30 plates