HiCrome™ Chromogenic Coliform Agar (CCA)

M1991I

Rrecommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition **

Ingredients	Grams/Litre
Tryptone#	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, dihydrate	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexamine ammonium salt, monohydrate	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	15.000

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Enzymatic digest of casein

Directions

Suspend 30.92 grams(the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Chromogenic Coliform Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water samples (1). The medium contains three chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxyl-β-D-galactopyranoside to form pink to red coloured colonies (3). The enzyme β-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl-β-D-glucuronic acid (2). Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, yeast extract, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (3).

The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples

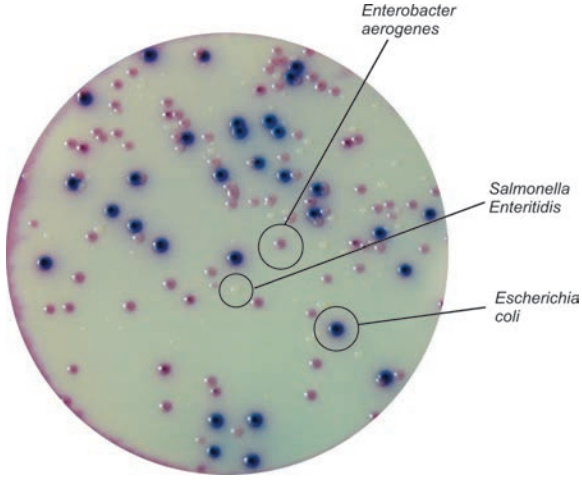
Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets



M1991I– HiCrome™ Chromogenic Coliform Agar (CCA)

HiCrome™ Single Streak Rapid Differentiation Series | HiCynth | Free from BSE/TSE/GMO

HiCrome™ Chromogenic Coliform Agar (CCA) (M1991I)is also available as HiCrome™ Chromogenic Coliform HiCynth™ Agar (CCA) (MCD1991I) wherein all animal/vegetable based nutrients are substituted with chemically defined nutrients.

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Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative hence *E.coli* species may show pink to red colonies.
2. Certain species of *Shigella* and *Salmonella* are ß-glucuronidase positive, hence they appear light blue to turquoise colonies.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : Light yellow coloured opalescent gel forms in Petri plates
Reaction : Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8 ± 0.2
Cultural Response : Cultural characteristics observed after an incubation at 34-38°C for 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii (43864) (00006*)	50-100	luxuriant	≥70%	pink to red
#Klebsiella aerogenes (13048) (00175*)	50-100	luxuriant	≥70%	pink to red
Escherichia coli (25922) (00013*)	50-100	luxuriant	≥70%	dark blue to violet
Enterococcus faecalis (29212) (00087*)	≥10^3	inhibited	0%	-
Pseudomonas aeruginosa (27853) (00025*)	50-100	luxuriant	≥70%	colourless

Key : * : corresponding WDCM Numbers
: Formerly known as *Enterobacter aerogenes*

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5, 6).

References

1. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part IMembrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
3. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.
4. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Ready Prepared Media			
Code	**Product Name**	**Usage**	**Packing**
Category : 90 mm Ready prepared Plates			
MP1991I	HiCrome™ Chromogenic Coliform Agar Plate (CCA Plate)	for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.	20 plts 50 plts
Category : 55 mm Scored Polystyrene Plates			
SP1991I	HiCrome™ Chromogenic Coliform Agar Plate	for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.	100 plts
Category : Drifilter™ Membrane Nutrient Pad			
MF034	Chromogenic Coliform Medium (without membrane filter)	for detection of *E.coli* and coliforms in water samples	20 plts 50 plts
MF034F	Chromogenic Coliform Medium w/sterile membrane filter	for detection of *E.coli* and coliforms in water samples	20 plts 50 plts