

HiCrome™ Chromogenic Coliform Agar Plate (CCA Plate)

MP1991I

Intended Use

Recommended 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/Amd 1:2016.

Composition**

ISO 9308-1:2014/Amd 1:2016 Specification - Chromogenic Coliform agar (CCA Agar)

| Ingredients | g / L |
|---|--------------|
| Enzymatic digest of casein | 1.000 |
| Yeast extract | 2.000 |
| Sodium chloride | 5.000 |
| Sodium dihydrogen phosphate, 2H ₂ O | 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 cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt) | 0.100 |
| IPTG (Isopropyl-β-D-thiogalactopyranoside) | 0.100 |
| Agar | 9.0 to 18.00 |
| Final pH (at 25°C) | 6.8±0.2 |

M1991I - HiCrome™ Chromogenic Coliform agar (CCA Agar)

| Ingredients | g / L |
|---|---------|
| Tryptone # | 1.000 |
| Yeast extract | 2.000 |
| Sodium chloride | 5.000 |
| Sodium dihydrogen phosphate, 2H ₂ O | 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 cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt) | 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

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Note: On storage at 2 to 8°C, white particles are likely to be observed, but on exposure at 35-37°C, the plates will be clear.

Principle And Interpretation

HiCrome™ Chromogenic Agar is a selective medium recommended by ISO for enumeration of *Escherichia coli* and coliform bacteria (1). The medium contains three chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxy 1-β-D-galactopyranoside to form pink to red coloured colonies (1). The enzyme β-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxy 1-β-D-glucuronic acid (1) 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, 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 sub-lethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (1). 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:

Processing (1)

Filtration: Filter 100ml of the sample using membrane filter. The minimum volume for filtration should be 10ml (or dilution) so that to ensure even distribution of the bacteria on the membrane filter.

Incubation and differentiation:

After filtration place the membrane filter on HiCrome™ Chromogenic Coliform agar (CCA Agar), ensuring that no air is trapped underneath, invert petri dish and incubate at 36°C ± 2 for 21 ± 3 hours. Examine the colony on membrane filters for color change.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. The media should be handled by trained personnel only. 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.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

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

Quality Control**Appearance**

Sterile HiCrome™ Chromogenic Coliform Agar in 90 mm disposable plates with smooth surface and absence of black particles/cracks/bubbles.

Colour of medium

Off white to Light yellow coloured medium

Quantity of Medium

25ml of medium in 90mm disposable plates.

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 36°C ± 2 for 21 ± 3 hours. Recovery is considered as 100% on TSA.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of Colony# |
|--|------------------|-----------|----------|---------------------|
| Productivity | | | | |
| <i>Escherichia coli</i> ATCC 25922 (00013)* | 50-100 | luxuriant | ≥70% | dark blue to violet |
| <i>Escherichia coli</i> ATCC 8739 (00012)* | 50-100 | luxuriant | ≥70% | dark blue to violet |
| <i>Citrobacter freundii</i> ATCC 43864 (00006)* | 50-100 | luxuriant | ≥70% | pink to red |
| ## <i>Klebsiella aerogenes</i> ATCC 13048 (00175)* | 50-100 | luxuriant | ≥70% | pink to red |
| Selectivity | | | | |
| <i>Enterococcus faecalis</i> ATCC 19433 (00009)* | ≥10 ⁴ | inhibited | - | - |
| <i>Enterococcus faecalis</i> ATCC 29212 (00087)* | ≥10 ⁴ | inhibited | - | - |
| Specificity | | | | |
| <i>Pseudomonas aeruginosa</i> ATCC 10145 (00024)* | 50-100 | luxuriant | - | colourless |

Key * : Corresponding WDCM numbers # : either on plate or membrane ## Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

On receipt store between 2-8°C 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 (2,3).

Reference

1. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I- Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014/Amd 1:2016.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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Disclaimer :

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