HiCrome™ ECC Selective Agar Base, Modified

M2056

Intended use

Recommended for detection of *Escherichia coli* and coliforms in water and food samples.

Composition**

Ingredients	g / L
Peptone	10.000
Sodium dihydrogen phosphate	2.200
Disodium hydrogen phosphate	2.700
Sodium chloride	5.000
Sodium pyruvate	1.000
L-Tryptophan	1.000
Sorbitol	1.000
Potassium nitrate	1.000
Sodium lauryl sulphate (SLS)	0.200
Chromogenic mixture	0.200
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.30 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ ECC Selective Agar, Modified is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (1,2). The chromogenic mixture contains two chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves one of the chromogen to form salmon to red coloured colonies (3). The enzyme ß-D-glucuronidase produced by *E.coli*, cleaves X-glucuronide, the other chromogen (4). Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. Addition of L-Tryptophan improves the indole reaction, thereby increasing the detection reliability. Peptone provide nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients for the organisms. Sodium pyruvate serves as a growth factor and sorbitol is the fermentable carbohydrate. Phosphates buffer the medium. The media formulation helps even sub lethally injured coliforms to recover and grow rapidly. Sodium lauryl sulphate inhibits gram-positive bacteria.

Type of specimen

Food and dairy samples; Water samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8). 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.

Limitations :

1. Certain strains of *E.coli* may exhibit salmon colour if they lack the ß-glucuronidase enzyme.
2. The intensity of the colour produced by *E.coli* and coliforms depends on the amount of enzyme produced by the organism.

3. Certain species of *Shigella* and *Salmonella* are ß-glucuronidase positive which may appear as light blue.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5 % Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.93% w/v aqueous solution at 25°C. pH : 7.00±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	dark blue
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	>=50%	pink
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=50%	pink
Citrobacter freundii ATCC 8090	50-100	luxuriant	>=50%	pink
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	

Key : (#) Formely known as *Enterobacter aerogenes*, (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 15-25°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 in order 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. 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 (9,10).

Reference

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