HiCrome™ ECC Selective HiVeg™ Agar Base

MV1294

Intended Use:

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

Composition**

Ingredients	g / L
HiVeg™ special peptone	6.000
HiVeg™ hydrolysate	3.300
Sodium dihydrogen phosphate	0.600
Disodium hydrogen phosphate	1.000
Sodium chloride	2.000
Sodium puruvate	1.000
L-Tryptophan	1.000
Sorbitol	1.000
Tergitol 7(Sodium heptadecyl sulphate)	0.150
Chromogenic mixture	0.430
Agar	10.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.48 grams 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. If desired, selective medium can be prepared by aseptically adding the rehydrated contents of 1 vial of ECC Selective Supplement (FD190) to previously cooled to 45-50°C sterile medium. Mix well and pour into sterile Petri plates. Medium may show haziness, but it does not affect the performance of the medium.

Principle And Interpretation

HiCrome™ ECC Selective Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (1,2). HiCrome™ ECC Selective HiVeg™ Agar is prepared by completely replacing animal based peptones with vegetable peptone to avoid BSE/TSE risks associated with animal peptones. 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* gives 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 special, 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 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (5). Addition of ECC Selective Supplement (FD190) helps to inhibit the accompanying heterogeneous microflora.

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

Food samples; Water samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards 6).

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. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 2.65% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	Indole production
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	>=50%	salmon to red	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	dark blue to violet	positive reaction
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good	40-50%	colourless	negative reaction
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%		
Shigella flexneri ATCC 29508	50-100	good	40-50%	light blue to turquoise	negative reaction
Citrobacter freundii ATCC 8090	50-100	luxuriant	>=50%	salmon to red (big)	negative reaction
Escherichia coli O157:H7 NCTC 12900	50-100	luxuriant	>=50%	salmon to red	positive reaction

Key : *Corresponding WDCM numbers
#- Formerly known as *Enterobacter aerogenes.*

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 (7,8).

Reference

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