



Technical Data

HiCrome ECC Selective Agar Base

M1294

HiCrome ECC Selective Agar is recommended for detection of *Escherichia coli* and coliforms in water and food samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	6.000
Casein enzymic hydrolysate	3.300
Sodium dihydrogen phosphate	0.600
Disodium hydrogen phosphate	1.000
Sodium chloride	2.000
Sodium pyruvate	1.000
L-Tryptophan	1.000
Sorbitol	1.000
Tergitol 7®	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 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 HiCrome ECC Selective Supplement (FD190) to previously cooled 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). The chromogenic mixture contains two chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves one of the chromogen to form salmon to red coloured colonies (3). The enzyme β-D-lucuronidase 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 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 (3). Addition of HiCrome ECC Selective Supplement (FD190) helps to inhibit the accompanying heterogenous 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.

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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	Indole production
Cultural Response					
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	≥50%	salmon to red (big)	negative reaction
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	≥50%	salmon to red	
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	≥50%	dark blue to violet	positive reaction
<i>Enterococcus faecalis</i> ATCC 29212	≥10 ³	inhibited	0%		
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good	40-50%	colourless	negative reaction
<i>Shigella flexneri</i> ATCC 29508	50-100	good	40-50%	light blue to turquoise	negative reaction
<i>Escherichia coli</i> O157:H7 NCTC 12900	50-100	luxuriant	≥50%	salmon to red	positive reaction

Storage and Shelf Life

Store dehydrated powder and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

- 1.Frampton E.W., Restaino L. and Blaszkowski N., 1988, J.Food Prof., 51:402.
- 2.Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand Sect. B, 84:245.
- 3.LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur > 102:267.
- 4.Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.

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