

For identification and differentiation of *E. coli* and Total coliforms

HiCrome™ ECC Selective Agar Base / Modified

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

M1294/
M2056

Composition **	M1294	M2056
Ingredients	Grams/Litre	Grams/Litre
Peptone, special	6.00	-
Peptone	-	10.00
Tryptone	3.30	-
Sodium dihydrogen phosphate	0.60	2.20
Disodium hydrogen phosphate	1.00	2.70
Sodium chloride	2.00	5.00
Sodium pyruvate	1.00	1.00
L-Tryptophan	1.00	1.00
Sorbitol	1.00	1.00
Tergitol-7® (Sodium heptadecyl sulphate)	0.15	-
Potassium nitrate	-	1.00
Sodium lauryl sulphate (SLS)	-	0.200
Chromogenic mixture	0.43	0.200
Agar	10.00	15.00
Final pH (at 25°C)	6.8 ± 0.2	7.00 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.48 grams of M1294 / 39.30 grams of M2056 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, selective medium can be prepared by aseptically adding the rehydrated contents of 1 vial of HiCrome™ ECC Selective Supplement (FD190) to M1294. 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

These are selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (1, 3). 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 (4). The enzyme β -D-glucuronidase produced by *E. coli*, cleaves X-glucuronide, the other chromogen (5). *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, Peptone, Tryptone and sodium pyruvate provide nitrogenous substances, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients for the organisms. Sorbitol is the fermentable carbohydrate. Phosphates buffer the medium. The media formulation helps even the sublethally injured coliforms to recover and grow rapidly. Tergitol in M1294 inhibits gram-

positive as well as some gram-negative bacteria other than coliforms (3). Sodium lauryl sulphate in M2056 inhibits gram-positive organisms. Addition of HiCrome™ ECC Selective Supplement (FD190) in M1294 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. Other gram negative bacteria forms colourless colonies, except some organisms which are β -glucuronidase positive. β -glucuronidase positive organisms gives light blue to turquoise colonies. Glucuronidase is present in 94–96% of *E. coli* strains and in some *Salmonella*, *Shigella* and *Yersinia* spp (2). To confirm *E. coli*, add a drop of Kovac's reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples ; Food samples

Specimen Collection and Handling

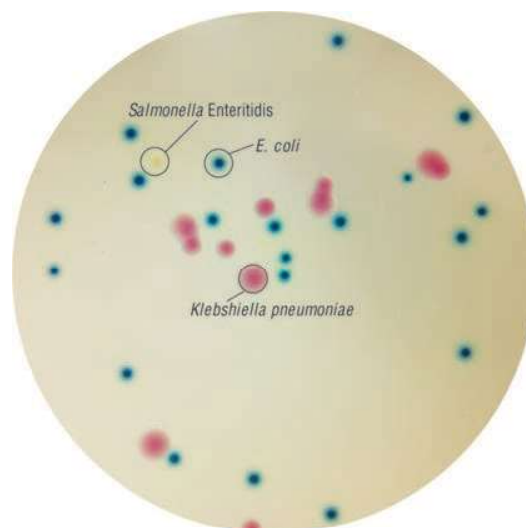
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7, 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



M1294 – HiCrome™ ECC Selective Agar Base

HiCromeVeg™
Single Streak Rapid Differentiation Series

Freedom from BSE / TSE worries

HiCrome™ ECC Selective Agar Base (M1294) is also available as HiCrome ECC Selective HiVeg™ Agar Base (MV1294) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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Limitations

1. β -glucuronidase is present in 97% of *E. coli* strains, however few *E. coli* may be negative.
2. Certain species of *Shigella* and *Salmonella* are β -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 recommended temperature.

Quality Control

Appearance of powder : Light yellow to pink (M1294) / Cream to yellow (M2056) coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.0% Agar gel (M1294)
Firm, comparable with 1.5% Agar gel (M2056)

Colour and Clarity of prepared medium : Light pink coloured (M1294) / Light yellow coloured (M2056), clear to slightly opalescent gel forms in Petri plates

Reaction : Reaction of 2.65% w/v aqueous solution of M1294 at 25°C. pH : 6.8 ± 0.2 .
Reaction of 3.93% w/v aqueous solution of M2056 at 25°C. pH : 7.00 ± 0.2 .

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

Organisms (ATCC)	Inoculum (CFU)	M1294 Growth w/FD190	M2056 Growth	Recovery	Colour of colony (M1294)	Colour of colony (M2056)
<i>Escherichia coli</i> (25922) (00013*)	50-100	good - luxuriant	good - luxuriant	$\geq 50\%$	dark blue to violet	dark blue
<i>Escherichia coli</i> O157:H7 (NCTC 12900)	50-100	luxuriant	-	$\geq 50\%$	salmon to red	-
<i>Klebsiella aerogenes</i> (13048) (00175*)	50-100	luxuriant	luxuriant	$\geq 50\%$	salmon to red	Pink
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	luxuriant	luxuriant	$\geq 50\%$	-	Pink
<i>Citrobacter freundii</i> (8090)	50-100	luxuriant	luxuriant	$\geq 50\%$	salmon to red (big)	Pink

<i>Salmonella</i> Enteritidis (13076) (00030*)	50-100	good	-	40-50%	colourless	-
<i>Shigella flexneri</i> (29508)	50-100	good	-	40-50%	light blue to turquoise	-
<i>Enterococcus faecalis</i> (29212) (00087*)	$\geq 10^3$	inhibited	inhibited	0%	-	-

Key : ● : positive reaction, confirmation of red colour around the colony by addition of Kovac's reagent (R008)

* : 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 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. 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 (9, 10).

References

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4. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur 102:267.
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6. 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
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10. 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 : HiTouch™ FlexiPlates			
FL022	HiTouch™ ECC Count Flexi Plate™	for enumeration (count) of <i>Escherichia coli</i> and coliforms.	50 plts
Category : DriFilter Membrane Nutrient Pad			
MF028	ECC Selective Medium (without Membrane Filter)	for detection and enumeration of total coliforms and <i>E. coli</i> based on chromogenic differentiation.	50 plts