

For Fluorogenic Identification of *E.coli*

HiCrome™ ECD Agar w/ MUG

Recommended for the detection of *Escherichia coli* in water and food samples by using a combination of chromogenic and fluorogenic substrate.

M1488

Composition **

Ingredients	Grams/Litre
Tryptone	20.00
Bile salts mixture	1.50
L-Tryptophan	1.00
Lactose	5.00
Sodium chloride	5.00
Dipotassium hydrogen phosphate	4.00
Potassium dihydrogen phosphate	1.50
Fluorogenic substrate	0.07
Chromogenic substrate	0.10
Agar	15.00

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.17 grams 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. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ ECD Agar w/ MUG is recommended for rapid detection of *Escherichia coli* by using a combination of chromogenic and fluorogenic substrates. The presence of *Escherichia coli* is indicated by blue coloured colony formation due to cleavage of chromogenic substrate. Fluorogenic substrate permits rapid detection of *Escherichia coli* when medium is observed for fluorescence using UV light (1, 2). Fluorogenic substrate also detects anaerogenic strains, which may not be detected in conventional procedure (1). It is hydrolysed by enzyme β -D-glucuronidase, possessed by *Escherichia coli* to yield a fluorescent end product. The reaction is indicated by a blue fluorescence under UV light. Tryptone provides nitrogenous, carbonaceous compounds, long chain amino acids and other essential nutrients. Lactose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. The medium has a strong buffering system to control the pH in the presence of fermentive action. The bile salt mixture inhibits gram-positive bacteria especially *Bacillus* species and faecal Streptococci.

Type of specimen

Food samples, Water samples

Specimen Collection and Handling

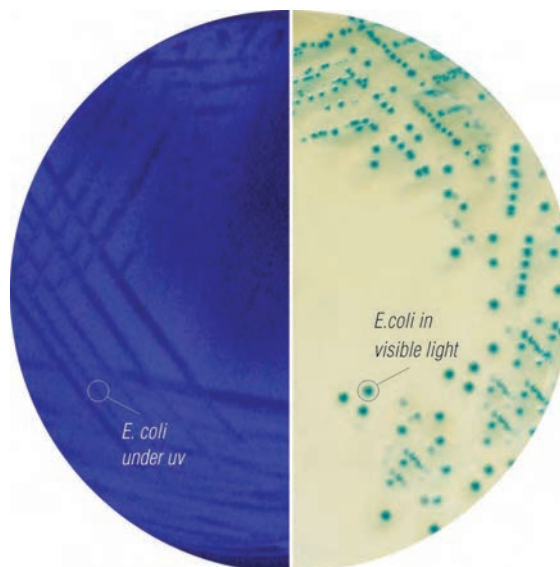
For food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).

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



M1488 HiCrome ECD Agar w/ MUG

HiCromeVeg™
Single Streak Rapid Differentiation Series

HiCrome™ ECD Agar w/ MUG (M1488) is also available as HiCrome™ ECD HiVeg™ Agar w/ MUG (MV1488) wherein all the animal origin nutrients have been replaced by vegetable base nutrients.

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Limitations

1. β -glucuronidase especially present in 97% of *Escherichia coli*, however few *E. coli* may be negative.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

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** : Cream to yellow coloured, homogeneous, free flowing powder.
- Gelling** : Firm, comparable with 1.5% Agar gel.
- Colour and Clarity of prepared medium** : Light amber coloured, clear gel forms in Petri plates.
- Reaction** : Reaction of 5.32% w/v aqueous solution at 25°C. pH : 7.0 ± 0.2
- Cultural Response** : Cultural characteristics observed after an incubation at 44-45°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Cololur of colony	Fluorescence under UV	Indole
<i>Escherichia coli</i> (25922) (00013*)	50-100	good	40-50%	bluish green	+	+
<i>Klebsiella pneumoniae</i> (13883) (00097*)	50-100	good	40-50%	colourless	-	-
<i>Staphylococcus aureus</i> subsp <i>aureus</i> (25923) (00034*)	$\geq 10^3$	inhibited	0%	-	-	-
<i>Enterococcus faecalis</i> (29212) (00087*)	$\geq 10^3$	inhibited	0%	-	-	-
<i>Pseudomonas aeruginosa</i> (27853) (00025*)	50-100	good	40-50%	colourless	-	-

Key : * : Corresponds to WDCM number

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 (5, 6).

References

1. Feng PCS and Hartman PAS, (1982), Appl. Environ. Microbiol. 43:132.
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4. 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
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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