

# Hicrome<sup>TM</sup> ECD Agar w/ MUG

# Intended use

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

## **Composition\*\***

Ingredients	<b>g</b> / L
Tryptone	20.000
Bile salts mixture	1.500
L-Tryptophan	1.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.500
Fluorogenic substrate	0.070
Chromogenic substrate	0.100
Agar	15.000
Final pH ( at 25°C)	$7.0{\pm}0.2$

\*\*Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 53.17 gram in 1000 ml purified/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<sup>TM</sup> 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 carbon, nitrogen substances, long chain amino acids, vitamins 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 fermentative 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 and processing as per guidelines (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.

## Limitations

- 1. ß-glucuronidase is present in 97% of E.coli strains, however few E.coli may be negative.
- 2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
- 3. Further biochemical and serological test needs to be carried out for confirmation.

## **M1488**

## **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 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

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pН
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6.80-7.20
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#### **Cultural Response**

Cultural characteristics observed after an incubation at 44-45°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony	Fluorescence (under uv)	Indole
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 <sup>4</sup>	inhibited	0%			
Escherichia coli ATCC 25922 (00013*)	50-100	good	40-50%	bluish-green	positive	positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	40-50%	colourless	negative	negative reaction
Pseudomonas aeruginosa ATCC 27853(00025*)	50-100	good	40-50%	colourless	negative	negative reaction
Staphylococcus aureus subsp. aureus ATCC	>=10 <sup>4</sup>	inhibited	0%			

25923 (00034\*)

Key : (\*) 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 (5,6).

## Reference

- 1. Feng PCS and Hartman PAS, (1982), Appl. Environ. Microbiol. 43:132.
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- 3. Robinson (1984), Appl. Environ. Microbiol., 48:285.
- 4. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
- 5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
- 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.

#### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia<sup>TM</sup> publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia<sup>TM</sup> Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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