



MUG EC O157 Agar

M1373

MUG EC O157 Agar is recommended for isolation and differentiation of enterohaemorrhagic *Escherichia coli* O157:H7 from foodstuffs, water and clinical samples by a fluorogenic method.

Composition**

Ingredients	Gms / Litre
Casein peptone	20.000
Meat extract	2.000
Yeast extract	1.000
Sorbitol	10.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Bromothymol blue	0.025
Sodium thiosulphate	2.000
Sodium deoxycholate	1.120
4-Methylumbelliferyl β -D-Glucuronide (MUG)	0.100
Agar	13.000
Final pH (at 25°C)	7.4 \pm 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.74 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 and pour into sterile Petri plates.

Principle And Interpretation

MUG EC O157 Agar is recommended (1) for isolation and enumeration of enterohaemorrhagic *Escherichia coli* (EHEC) from foodstuffs, water and clinical samples based on sorbitol utilization and formation of beta-glucuronidase enzyme. The enterohaemorrhagic *E. coli* O157:H7 strains produce toxins, which can result in life threatening extra intestinal complications in the form of the hemolytic uremic syndrome and thrombotic-thrombocytopenic purpura. Due to severe clinical implications, the isolation and detection of *E. coli* O157:H7 strains are of importance.

Sodium deoxycholate inhibits the growth of gram-positive microbes. Sorbitol provides carbon and energy source. Bromothymol blue is the pH indicator. Microorganisms utilizing sorbitol exhibit yellow colonies whereas sorbitol-negative strains (such as *E. coli* O157:H7) grow as greenish colonies. Hydrogen sulphide production is detected as black-brown colony colouration due to presence of sodium thiosulphate and ferric ammonium citrate. Thus *Proteus mirabilis* having similar biochemical characteristics as that of *E. coli* O157:H7 can easily be differentiated. 4-Methylumbelliferyl b-D-glucuronide (MUG) is converted into 4-methylumbelliferone by beta-D-glucuronidase-forming pathogens. 4-methylumbelliferone fluoresces under UV light. All commensal *E. coli* produce beta-glucuronidase. *E. coli* O157:H7 is not capable of forming b-glucuronidas, thus when exposed under long-wave UV light, no fluorescence is observed. The plates were exposed to ammonia fumes to increase fluorescence as suggested by Freir and Hartman (2).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Bluish green coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.47% w/v aqueous solution at 25°C. pH : 7.4 \pm 0.2

pH

7.20-7.60

Cultural Response

M1373: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Fluorescence (under UV)*
Cultural Response					
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	>=50%	yellow	negative
<i>Escherichia coli</i> O157:H7	50-100	luxuriant	>=50%	colourless	negative
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	>=50%	yellow	positive
<i>Enterococcus faecalis</i> ATCC 19433	>=10 ³	inhibited	0%		
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	>=50%	brown, may show black colouration(H ₂ S production)	negative
<i>Salmonella</i> Typhimurium ATCC 14028	50-100	luxuriant	>=50%	yellow w/black centre	negative

Key :* - Fluorescence can be visualized on addition of NaOH solution or exposure to ammonia fumes.

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Szabo R. A., Todd E. C., Jean A., 1986, J. Food Prot., 10:768-772.
2. Freir T.A. and Hartman P.A. (1987) Appl. Env. Microbiol. 53. 1246-1250

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