



ECD MUG Agar

M1358

ECD MUG Agar is recommended for demonstrating the presence of *Escherichia coli* by fluorescence in UV and positive indole test while inhibiting accompanying intestinal flora.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Lactose	5.000
Sodium chloride	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	4.000
Monopotassium dihydrogen phosphate	1.500
Tryptophan	1.000
4-Methylumbelliferyl β-D-Glucuronide (MUG)	0.070
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.07 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. Mx well and pour into sterile Petri plates.

Principle And Interpretation

EC Medium developed by Hajna and Perry (1) to improve the methods for the detection of coliforms and *Escherichia coli*. Feng and Hartman (2) developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-gluconide (MUG) in to Lauryl Tryptose Broth. E.C Medium with MUG is prepared according to the formula specified by the U.S Environmental Protection Agency (3) and Standard methods for water and food testing (4, 5).

Casein enzymic hydrolysate provides the nitrogen, vitamins and amino acids in EC medium with MUG. Lactose is the carbon source in this medium. Bile salts mixture is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium phosphate and mono potassium phosphate are buffering agents. Sodium chloride maintains the osmotic balance of the medium. *E.coli* produces the enzyme glucuronidase that hydrolysis MUG to yield a fluorogenic product that is detectable under long wave (366 nm) UV light. Tryptophan (M1358) serves as the substrate for indole reaction.

The water sample is filtered through filter membranes, which are then placed on ECD MUG Agar and incubated overnight. After incubation observe for the presence of fluorescence under UV light. Lay a drop of Kovacs Indole reagent (R008) on the colonies. Indole positive colonies form a red zone around the colony. MUG positive and indole positive colonies are enumerated as *E. coli*.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

M1358: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Indole production	Fluorescence (under 366nm)
Cultural Response <i>Enterobacter aerogenes</i> ATCC 13048	50-100	good-luxuriant	>=50%	negative reaction	negative
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	>=50%	positive reaction, red zone around the colony	positive
<i>Staphylococcus aureus</i> ATCC 25923	>=10 ³	inhibited	0%		

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. Hajna and Perry 1943. Am J Public Health 33:550.
2. Feng P. C. and Hartman P. A., 1982 Appl. Environment Microbiol 43:1320.
3. Federal Register 1991. National primary drinking water regulation analytical techniques, coliform bacteria. Fed Register. 56:636.
4. Clesceri L. S., Greenberg A. E. and Eaton A. D. (ed.), 1998 Standard methods for the examination of water and waste water; 20th ed. American Public Health Association, Washington, D.C.
5. U.S Food and Drug Administration. 1995, Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.

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