EC Medium with MUG
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NCM0107

Intended Use
EC Medium w/ MUG is used for the fluorogenic detection of *Escherichia coli* in a laboratory setting. EC Medium w/ MUG is not intended for use in the diagnosis of disease or other conditions in humans.

Description
EC Medium was developed by Hajna and Perry in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria is inhibited by the bile salts, while growth of *E. coli* is enhanced by its presence. EC Medium w/ MUG is the same formula as EC Medium, with the addition of 4-methylumbelliferyl-β-D-glucuronide. Feng and Hartman developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) into Lauryl Tryptose Broth at a final concentration of 100 μg/mL. Moburg determined the amount of MUG could be reduced to a final concentration of 50 μg/mL without adversely affecting results.

EC Medium w/ MUG is prepared according to the formula specified by US EPA and methods for water and food testing.

Typical Formulation

Tryptose	20.0 g/L
Lactose	5.0 g/L
Bile Salts	1.5 g/L
Dipotassium Phosphate	4.0 g/L
Monopotassium Phosphate	1.5 g/L
Sodium Chloride	5.0 g/L
4-Methylumbelliferyl-β-D-Glucuronide	0.05 g/L

Final pH: 6.9 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Dissolve 37 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Distribute into tubes containing inverted Durham tubes.
4. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is brilliant to clear and yellow, with none to light precipitate.

CULTURAL RESPONSE
With reference to the FDA/BAM Chapter 4 Enumeration of Escherichia coli and the Coliform Bacteria (update 2/2013) and APHA Standard Methods for the Examination of Water and Wastewater 20th Edition Section 9221: The medium was prepared according to label directions and inoculated with the organisms listed below. Two inoculation methods can be followed:

- Direct inoculation of test strains into EC Medium with MUG with incubation at 35± 2°C and at 44.5±0.2°C. Incubate cultures in an aerobic atmosphere at indicated temperature and then examine for growth and gas at 24 ± 2 hours; if no gas re-incubate to 48 ± 3 hours to confirm gas.

- Pre-enrich test strains in LTB (Lauryl Tryptose Broth) at 35± 2°C. If no growth/gas after 24 hours, re-incubate up to 48 hours and transfer a loopful of LTB enriched culture to EC Medium w/ MUG and incubate in a water bath at 44.5 aerobically. Examine for growth, gas and fluorescence at 24 ± 2 hours.

			EXPECTED RESULTS			
MICRO-ORGANISM	**ATCC**	**APPROX. INOCULUM (CFU)**	**Direct inoculation into EC w MUG at 35C/44.5C**		**LTB pre-enrichment at 35C; EC w MUG at 44.5C**	
			Growth	Gas/Fluor	Growth	Gas/Fluor
Enterococcus faecalis	29212	10^3	Inhibited	N/A	Inhibited	N/A
Escherichia coli	25922	10-300 for 35C; ~10^3 for 44.5C; 10-100 for LTB pre-enrichment	Fair to excellent	Weak pos gas; pos fluor w/ growth	Good to Excellent	Pos gas; pos fluor

Test Procedure
Refer to appropriate references for specific procedures using EC Medium w/ MUG.

Results
Following incubation observe tubes for growth, production of gas, and fluorescence. Positive gas production is demonstrated by displacement of the medium from the fermentation vial. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light. Typical strains of *E. coli* are positive for both gas production and fluorescence. Non-*E.coli* coliforms that grow may produce gas, but will not exhibit fluorescence.

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

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Limitations of the Procedure

1. Some strains may be encountered that grow poorly or fail to grow on this medium.
2. Strains of *E. coli* that fail to grow in EC Medium w/ MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered. Strains of *Salmonella*, *Shigella* and *Yersinia* that glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters, e.g., gas production, growth at 44.5°C.
3. Shellfish samples may contain endogenous glucuronidase which may cause false positive fluorescence reactions at the presumptive stage. It has been recommended to use EC Medium with MUG in the confirmatory stage for this sample group.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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5. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 2017. Standard methods for the examination of water and wastewater, 23rd ed., American Public Health Association, Washington, D.C.
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