EC MEDIUM W/ MUG (7361)

Intended Use
EC Medium w/ MUG is used for the fluorogenic detection of *Escherichia coli.*

Product Summary and Explanation
EC Medium was developed by Hajna and Perry[1] 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[2] 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[3] 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[4] and methods for water and food testing.[5,6]

Principles of the Procedure
Tryptose provides nitrogen, vitamins, and amino acids in EC Medium w/ MUG. Lactose is the carbon source. Bile Salts Mixture is the selective agent against non-fecal Gram-positive bacteria. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Incubation at 44.5°C provides additional selectivity.

E. coli produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light. The addition of MUG to EC Medium provides another criterion, along with growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

Formula / Liter
Tryptose ..... 20 g
Lactose ..... 5 g
Bile Salts Mixture ..... 1.5 g
Dipotassium Phosphate ..... 4 g
Monopotassium Phosphate ..... 1.5 g
Sodium Chloride ..... 5 g
4-Methylumbelliferyl-β-D-Glucuronide ..... 0.05 g
Final pH: 6.9 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
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.

Expected Cultural Response: Cultural response in EC Medium w/ MUG incubated aerobically in a 44.5°C water bath and examined for growth after 24 ± 2 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results		
		Recovery	Gas	Fluorescence (MUG)
Enterococcus faecalis ATCC® 29212	~ 10^3	Inhibited	--	--
Escherichia coli ATCC® 25922	~ 10^3	Growth	+	+

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures using EC Medium w/ MUG.[4-6]

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.

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.

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.

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.[7]

Packaging

EC Medium w/ MUG	**Code No.**	**7361A**	**500 g**
		7361B	**2 kg**
		7361C	**10 kg**

References

1. **Hajna and Perry.** 1943. Am J. Public Health. **33:**550.
2. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43:**1320-1329.
3. **Moberg, L. J.** 1985. Fluorogenic assay for rapid detection of *Escherichia coli* in food. Appl. Environ. Microbiol. **50:**1383-1387.
4. **Federal Register.** 1991. National primary drinking water regulation; analytical techniques; coliform bacteria. Fed. Regist. **56:**636-643.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed., American Public Health Association, Washington, D.C.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Koburger and Miller.** 1985. J. Food Prot. 48:244

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