



NUTRIENT AGAR with MUG

INTENDED USE

Nutrient Agar with MUG is used for detecting and enumerating *Escherichia coli* in water.

SUMMARY AND EXPLANATION

Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination. Feng and Hartman developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl- β -D-glucuronide (MUG) at a final concentration of 100 μ g/mL into Lauryl Tryptose Broth. Nutrient Agar is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration procedure by transferring the membrane from a total-coliform or fecal coliform positive sample to a Nutrient Agar substrate containing 4-methylumbelliferyl- β -D-glucuronide (MUG).

Mates and Shaffer used the membrane filter-Endo Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer. *E. coli* was recovered at a rate of 98% with no false-positive results.

Nutrient Agar with MUG is prepared according to the formula specified by the U.S. Environmental Protection Agency and published in *Standard Methods for the Examination of Water and Wastewater*.

PRINCIPLE

Beef extract and peptone are sources of nitrogen, vitamins, carbon and amino acids. Agar is the solidifying agent. The substrate, MUG (4-methylumbelliferyl- β -D-glucuronide), produces a blue fluorescence when hydrolyzed by the enzyme β -glucuronidase, which is produced by most *E. coli*.

REAGENTS (FORMULA)

Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g
MUG (4-Methylumbelliferyl- β -D-glucuronide)	0.1	g
Deionized Water	1000.0	ml

PROCEDURE

Follow the methods and procedures for water testing using m Endo Agar LES in standard methods. After incubation on m Endo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at $35 \pm 2^\circ\text{C}$. Expose the filter surface to long-wave UV light.

EXPECTED RESULTS

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony under long-wave (approximately 366 nm) UV light.

Typical strains of *E. coli* (red with a green metallic sheen on m Endo Agar LES) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
<i>Enterobacter aerogenes</i> ATCC 13048	$35 \pm 2^\circ\text{C}$ for 4-24 hours	Growth, Fluorescence Negative
<i>Escherichia coli</i> ATCC 25922	$35 \pm 2^\circ\text{C}$ for 4-24 hours	Growth, Fluorescence Positive

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BIBLIOGRAPHY

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