IFU

Instructions for Use

NUTRIENT AGAR WITH MUG

Cat. no. G114	Nutrient Agar with MUG, 15x60mm Plate, 11ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Nutrient Agar with MUG is recommended for detecting and enumerating *Escherichia coli* in water samples.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Escherichia coli is a member of the fecal coliform group of bacteria and its presence in water samples is indicative of fecal contamination.(1) Feng and Hartman developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) at a final concentration of 100µg/ml into Lauryl Tryptose Broth.(2) Nutrient Agar is modified the same way with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration method by transferring the membrane from a total coliform or fecal coliform positive sample to a Nutrient Agar substrate containing MUG.(1) Mates and Shaffer used the membrane filter-Endo LES Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer.(3) *E. coli* was recovered at a rate of 98% with no false-positive results.

The formulation of Nutrient Agar is composed of peptone, beef extract and agar. Nutrients necessary for the replication and growth of a large number of nonfastidious microorganisms are provided by the simple formulation. Water soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts are present in the beef extract.(10) Peptone supplies the principle source of organic nitrogen in the form of amino acids and long-chained fatty acids. 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*.

FORMULA

Ingredients per liter of deionized water:*

Peptone	5.0gm
Beef Extract	3.0gm
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.1gm
Agar	15.0gm

Final pH 6.8 +/- 0.3 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store media in plates at 2-8°C. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Sample collection: Consult listed references for information on sample collection and procedures for water testing.[1,11]

Membrane filter-Endo LES Agar method, followed by incubation on Nutrient Agar with MUG:

1. Follow the methods and procedures for water testing using m Endo LES Agar described in Standard Methods.[1]

2. After incubation on m Endo LES Agar for 24 hours (Cat. no. G28), aseptically transfer the membrane to Nutrient Agar with MUG.[1,3,11]

3. Incubate plates at 35 +/- 0.5°C for 4 hours.

4. Expose the filter surface to long-wave (approximately 366nm) UV light, preferably containing a 6-Watt bulb.

INTERPRETATION OF RESULTS

Examine plates for colonies showing typical colony morphology, color, and fluorescence after incubation. Positive MUG reactions exhibit a blue fluorescence around the periphery of the colony under long-wave UV light.

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect colony fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect colony fluorescence. See 'CAUTION' above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over isolated colonies on the plate, approximately 3 to 4 inches (7 to 10cm) away.
3. Isolated colonies of *E. coli* will fluoresce a blue glow.
4. Only well isolated colonies will fluoresce. Colonies in areas of confluent growth will not.
5. Fluorescence will fade over time.

Typical strains of *E. coli* (red with a green metallic sheen on m Endo LES Agar, Cat. no. G28) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Glucuronidase-negative strains of *E. coli* have been encountered.[5-7] Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.[3]

Strains of *Salmonella* and *Shigella* species that produce β-glucuronidase may infrequently be encountered.[8] These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, lactose fermentation or growth at 44.5°C.

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, UV lamps, applicator sticks, incinerators, handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with compatible UV lamp (Cat. no. EA160), and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth with blue fluorescence under long-wave UV light
Enterobacter aerogenes ATCC® 13048	A	18-24hr	35°C	Aerobic	Growth without fluorescence under long-wave UV light

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Nutrient Agar with MUG should appear clear to slightly opalescent, and light amber in color.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

2. Feng and Hartman. 1982. *Appl. Environ. Microbiol.*; 43:1320.

3. Mates and Shaffer. 1989. *J. Appl. Bacteriol.*; 67:343.

4. Federal Register. 1991. *Fed. Regist.*; 56:636.

5. Chang, Brill and Lum. 1989. *Appl. Environ. Microbiol.* ; 55:335.

6. Hansen and Yourassowsky. 1984. *J. Clin. Microbiol.* ; 20:1177.

7. Kilian and Bulow. 1976. Sect. B *Acta Pathol. Microbiol. Scand.* ; 84:245.

8. Damare, Campbell and Johnston. 1985. *J. Food Sc.*; 50:1736.

9. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria,* Vol. I. Williams & Wilkins, Baltimore, MD.

10. Pelczar, Chan and Kreig. 1986. *Microbiology* , 5th ed. McGraw-Hill Book Company, New York, NY.

11. Environmental Protection Agency. 2005. *Manual for the Certification of Laboratories Analyzing Drinking Water*, EPA-815-R-05-004. Office of Ground Water and Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

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