IFU

Instructions for Use

EC BROTH WITH MUG AND DURHAM TUBE

Cat. no. K64	EC Broth with MUG and Durham Tube, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K18	EC Broth with MUG and Durham Tube, 20x125mm Tube, 13ml	20 tubes/box

INTENDED USE

Hardy Diagnostics EC Broths with MUG and Durham Tube is recommended for the detection of *Escherichia coli* in water and food samples by fluorogenic means.

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

SUMMARY

EC Broth with MUG (4-methylumbelliferyl-beta-D-glucuronide) is composed of the same basal formula as EC Broth developed by Hajna and Perry.[8] The medium consists of a buffered lactose broth with casein peptones, bile salts, and MUG.

Lactose provides fermentable carbohydrate for the growth of coliforms. Casein peptones provide a source of nutrients. The bile salts serve as inhibitory agents toward gram-positive cocci and spore-formers, particularly fecal streptococci and bacilli. The pH of the medium is maintained by the presence of a strong potassium buffering system.

The addition of MUG, a fluorogenic compound, allows for the rapid detection of *E. coli* when the medium is observed for fluorescence using a long-wave (366nm) UV light source.[7,9] Anaerogenic strains of *E. coli* can also be detected through the use of MUG.[7]

The detection of *E. coli* with MUG is based on the ability of beta-glucuronidase, an enzyme possessed by most *E. coli* strains, to hydrolyze 4-methylumbelliferyl-beta-D-glucuronide. The hydrolysis of MUG by *E. coli* yields 4-methylumbelliferone, a fluorescent end product.[7,9] Development of fluorescence allows the detection of *E. coli* in pure or mixed cultures within 4 to 24 hours following inoculation and incubation of EC Broth with MUG.

Studies conducted by Feng and Hartman revealed beta-glucuronidase activity in 96% of *E. coli*, 100% of enterotoxigenic *E. coli*, 17% *Salmonella* spp. and 40% of *Shigella* spp.[7]

EC Broth is recommended by the American Public Health Association (APHA) for the detection and enumeration of coliform organisms in foods, waters, and wastewater.[1,2]

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	20.0gm
Lactose	5.0gm

Sodium Chloride	5.0gm
Dipotassium Phosphate	4.0gm
Monopotassium Phosphate	1.5gm
Bile Salts Mixture	1.5gm
MUG	50.0mg

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store the product at 2-8ºC. Products should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, 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.[1-5]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning inoculation procedures.[1-5]

Incubate tubes in a 44.5 +/- 0.2ºC. waterbath for water and wastewater. Incubate tubes in a waterbath at 45.5 +/- 0.2ºC. for foods other than shellfish.

INTERPRETATION OF RESULTS

Production of turbidity and gas within 24 +/- 2 hours of incubation without fluorescence is considered positive evidence of fecal coliforms in water and wastewater. Presence of growth and the production of a bright blue fluorescence under a long-wave UV light (with or without the production of gas) is considered positive for the presence of *E. coli*. Absence of gas production within 24 +/- 2 hours is considered a negative test for fecal coliforms.

Production of turbidity and gas within 48 +/- 2 hours of incubation without fluorescence is considered considered positive evidence of fecal coliforms in foods other than shellfish. Presence of growth and the production of a bright blue fluorescence (with or without gas production) under a long-wave UV light is considered positive for the presence of *E. coli*. Absence of gas production within 48 +/- 2 hours is considered a negative test for fecal coliforms.

Consult listed references for detailed results for the enumeration of coliforms using EC Broth with MUG.

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect broth 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 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect broth fluorescence. See 'CAUTION' above regarding inexpensive 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 the tubes, approximately 3 to 4 inches (7 to 10cm) away.
3. The presence of *E. coli* will fluoresce a blue glow.
4. Fluorescence will fade over time.

LIMITATIONS

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

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.

It may be necessary to invert the tube prior to inoculation if bubbles are trapped in the durham tube. Trapped bubbles that are not released may lead to false-positive results.

Turbidity alone is not indicative of a positive test for the presence of fecal coliforms; turbidity with gas production is considered a positive test for fecal coliforms. Fluorescence may be observed in anaerogenic *E. coli*.

An uninoculated tube of media should be included as a batch control to detect weak autofluorescence of the medium.

Some strains of *Shigella* and *Salmonella* produce beta-glucuronidase which may result in false interpretation of test results.

The presence of streptococci in the test sample may lead to false-positive results.

Enterobacter aerogenes will not produce gas, and growth may be reduced, when incubated at 44.5°C.

False-positive results may occur when testing oysters. Oysters produce glucuronidase which interferes with the

accuracy of the assay. When testing oyster samples, it is recommended that an enrichment step in Lauryl Sulfate Broth be performed prior to inoculation of the test sample to EC Broth with MUG. The preenrichment step dilutes the glucuronidase from the oysters and decreases the possibility of false-positive results.

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

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC®25922**	A	24hr	35°C	Aerobic	Growth; turbidity with gas production and fluorescence under a long-wave UV light
Enterobacter aerogenes ATCC® 13048**	A	24hr	35°C	Aerobic	Growth; turbidity with gas and no fluorescence
Enterococcus faecalis ATCC® 29212**	B	24hr	35°C	Aerobic	Inhibited

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

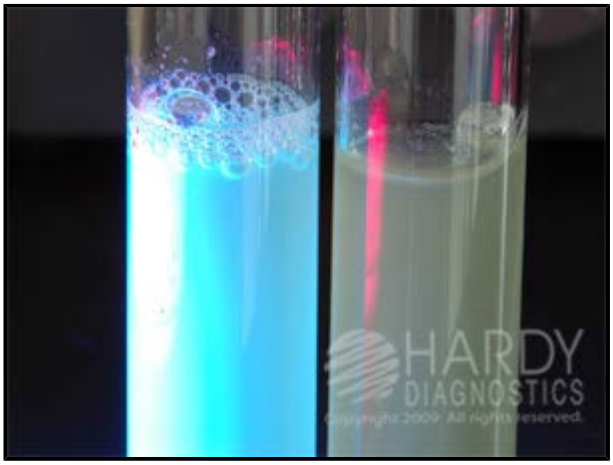
**Recommended QC strains.

USER QUALITY CONTROL

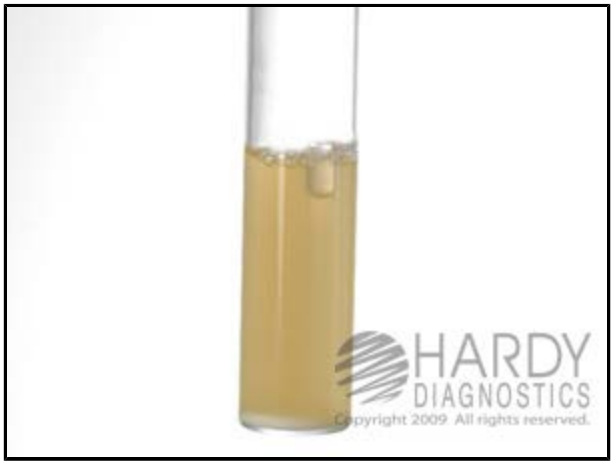
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

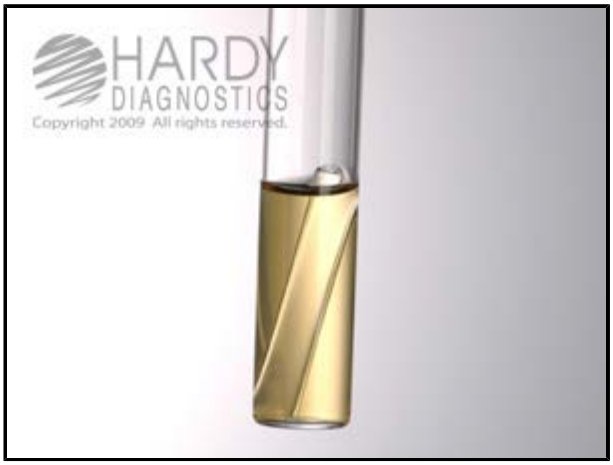
Both EC Broths with MUG and Durham Tube should appear clear, and colorless to light amber in color.



Escherichia coli (ATCC® 25922) (LEFT) and *Enterobacter aerogenes* (ATCC® 13048) (RIGHT) growing in EC Broth with MUG (Cat. no. K18) under UV light. Incubated aerobically for 24 hours at 35ºC.



Escherichia coli (ATCC® 25922) growing in in EC Broth with MUG (Cat. no. K18) under ambient light. The bubble in the Durham tube indicates gas production. Incubated aerobically for 24 hours at 35ºC.



Enterococcus faecalis (ATCC® 29212) growth inhibited in EC Broth with MUG (Cat. no. K18) under ambient light. Incubated aerobically for 24 hours at 35ºC.

REFERENCES

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

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods.* APHA, Washington, D.C.

3. American Public Health Association. *Standard Methods for the Examination of Dairy Products.* APHA, Washington, D.C.

4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

5. Association of Official Analytical Chemists. *Official Methods of Analysis.* AOAC, Washington, D.C.

6. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA.

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

8. Hajna and Perry. 1943. *Am. J. Public Health;* 33:550.

9. Robison. 1984. *Appl. Environ. Microbiol.;* 48:285.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]