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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

CRITERION™ EC MEDIUM WITH MUG

Cat. no. C5680	CRITERION™ EC Medium with MUG	74gm
Cat. no. C5681	CRITERION™ EC Medium with MUG	500gm
Cat. no. C5682	CRITERION™ EC Medium with MUG	2kg
Cat. no. C5683	CRITERION™ EC Medium with MUG	10kg
Cat. no. C5684	CRITERION™ EC Medium with MUG	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ EC Medium with MUG is recommended for the detection of *Escherichia coli* in water and food samples by fluorogenic means.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

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

Lactose provides fermentable carbohydrate for the growth of coliforms. Pancreatic digest of casein provides 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. Once hydrolyzed, the substrate yields 4-methylumbelliferone, a fluorescent end product.[7,9] Development of fluorescence allows the detection of *E. coli* in pure or mixed cultures within 4-24 hours following inoculation and incubation of EC Medium 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 Medium with MUG is recommended by the American Public Health Association (APHA) for the detection and enumeration of coliform organisms in foods, waters and wastewaters.[1,2]

FORMULA

Gram weight per liter:	37.0gm/L
Pancreatic Digest of Casein	20.0gm
Lactose	5.0gm
Sodium Chloride	5.0gm
Dipotassium Phosphate	4.0gm
Monopotassium Phosphate	1.5gm
Bile Salts No. 3	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

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light beige.

Store the prepared culture media at 2-30ºC.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 37.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Warm slightly to dissolve completely.

3. Dispense into test tubes containing inverted fermentation vials (durham tubes).

4. Autoclave at 121ºC. for 15 minutes.

PROCEDURE AND INTERPRETATION OF RESULTS

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 a 366nm or 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 isolated colonies on the plate, 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.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

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 coliforms; turbidity with gas production is considered a positive test. Fluorescence may be observed in anaerogenic *E. coli.*

Strains of *E. coli* that fail to grow in EC Medium with MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered.

Strains of *Salmonella*, *Shigella*, and *Yersinia* that produce glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, growth at 44.5ºC.

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

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 Medium 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 blue fluorescence under a long-wave UV light source
Enterobacter aerogenes ATCC® 13048	A	24hr	35°C	Aerobic	Growth; turbidity with gas production, without fluorescence
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibited

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

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ EC Medium with MUG powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear clear, 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. 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. *Bacteriological Analytical Manual* (BAM), 2002. Association of Official Analytical Chemists International, Gaithersburg, MD.

5. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed. 1990. AOAC, Arlington, VA.

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.

10. Federal Register. 1991. National primary drinking water regulation; analytical techniques; coliform bacteria. *Fed Regist.*; 56:636-643.

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

HDQA 2207B [D]