IFU

Instructions for Use

CRITERION™ MACCONKEY AGAR WITH MUG

Cat. no. C6110	CRITERION™ MacConkey Agar with MUG	100gm
Cat. no. C6111	CRITERION™ MacConkey Agar with MUG	500gm
Cat. no. C6112	CRITERION™ MacConkey Agar with MUG	2kg
Cat. no. C6113	CRITERION™ MacConkey Agar with MUG	10kg
Cat. no. C6114	CRITERION™ MacConkey Agar with MUG	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ MacConkey Agar with MUG is used for the presumptive identification of *Escherichia coli*.

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

Trepeta and Edberg modified MacConkey Agar by the incorporation of 4-methylumbelliferyl-beta-D-glucuronide (MUG); the resulting medium allowed the authors to presumptively identify *Escherichia coli* from the primary plating medium within five minutes.[8] The above reaction is possible because most strains of *E. coli* (96-97%) produce glucuronidase, an enzyme that hydrolyzes MUG to 4-methylumbelliferone.[6] This compound fluoresces blue under long-wave ultraviolet light (366nm). The addition of MUG to the formulation allows beta-glucuronidase-positive strains of *E. coli* to fluoresce blue-green when examined under this wavelength of UV light.

FORMULA

Gram weight per liter:	50.0gm/L
Pancreatic Digest of Gelatin	17.0gm
Lactose	10.0gm
Sodium Chloride	5.0gm
Pancreatic Digest of Casein	1.5gm
Peptic Digest of Animal Tissue	1.5gm
Bile Salts No. 3	1.5gm
MUG	150.0mg

Neutral Red	30.0mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.1 +/- 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 beige.

Store the prepared culture media at 2-8ºC.

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 50.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC. Dispense into sterile containers as desired.

PROCEDURE AND INTERPRETATION OF RESULTS

Examine plates for colonies showing typical colony morphology, color, and fluorescence after incubation. *E. coli*

colonies will be pink under ambient light. Positive MUG reactions exhibit a blue-green fluorescence around the periphery of the colony under long-wave UV light. Typical strains of *E. coli* exhibit blue-green fluorescence on MacConkey Agar with MUG. Non-*E. coli* coliforms may may be pink, but do not fluoresce.

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-green glow.
4. Only well isolated colonies will fluoresce. Colonies in areas of confluent growth will not.
5. Fluorescence will fade over time.

LIMITATIONS

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.

Not all strains of *Escherichia coli* ferment lactose or produce beta-glucuronidase. Some strains of *Salmonella* and *Shigella* produce beta-glucuronidase and will fluoresce. A small percentage of *Yersinia* and streptococci have been reported to fluoresce.[(5)]

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

Additional biochemical and/or serological tests are necessary for definitive identification.

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	Pink colonies; fluoresce blue-green under UV light (366nm)
Shigella sonnei ATCC® 9290	A	24hr	35°C	Aerobic	Clear colonies; The colonies fluoresce blue-green under UV light (366nm)
Proteus mirabilis ATCC® 12453	A	24hr	35°C	Aerobic	Clear colonies; no fluorescence under UV light (366nm)

* 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™ MacConkey Agar with MUG powder should appear homogeneous, free-flowing, and beige in color. The prepared media should appear opalescent, and reddish-purple in color.

Note: This medium contains 4-methylumbelliferyl-beta-D-glucuronide (MUG). Most strains of *E. coli* possess an enzyme which breaks down MUG to a compound that fluoresces under a long-wave (366nm) ultra violet lamp.

REFERENCES

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3. Feng, P.C.S. and P.A. Hartman. *Appl. Envir. Microbiol.*; 43:1982. p. 1320-1329.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

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8. Trepeta, R.W. and S.C. Edberg. *J. Clin. Microbiol.*; 19:1984. p. 172-174.

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

HDQA 2207B [D]