IFU

Instructions for Use

CRITERION™ BRILLIANT GREEN BILE BROTH WITH MUG

Cat. no. C5280	CRITERION™ Brilliant Green Bile Broth with MUG	80gm
Cat. no. C5281	CRITERION™ Brilliant Green Bile Broth with MUG	500gm
Cat. no. C5282	CRITERION™ Brilliant Green Bile Broth with MUG	2kg
Cat. no. C5283	CRITERION™ Brilliant Green Bile Broth with MUG	10kg
Cat. no. C5284	CRITERION™ Brilliant Green Bile Broth with MUG	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Brilliant Green Bile Broth with MUG is a selective medium recommended for the detection of coliforms in water, sewage, dairy products and other samples.

The addition of 4-methylumbelliferyl-beta-D-glucuronic acid (MUG) reagent detects *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

Brilliant Green Bile Broth with MUG is a selective medium for the detection of coliforms in water, foods, dairy products and other materials.[1,4,5] Bile and brilliant green inhibit growth of most organisms other than coliforms. Coliform detection is based on lactose-fermentation with corresponding accumulation of gas within inverted fermentation vials. This medium is formulated according to APHA specifications.[4,5] The addition of MUG reagent makes this medium specific for *Escherichia coli*. Most strains (96-97%) of *E. coli* produce glucuronidase. This enzyme hydrolyzes MUG reagent to 4-methylumbelliferone, a fluorescent compound, detected by (long-wavelength) UV light. Fluorescence produced in the broth is highly specific for *E. coli*.

FORMULA

Gram weight per liter:	40.0gm/L
Oxbile (Oxgall)	20.0gm
Pancreatic Digest of Gelatin	10.0gm
Lactose	10.0gm
MUG Reagent	50.0mg

Brilliant Green	13.3mg

Final pH 7.2 +/- 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 greenish-tan.

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 40.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat as necessary to dissolve completely. Avoid overheating.

3. Dispense into tubes containing durham tubes.

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

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

Examine plates for colonies showing typical morphology and color. Use a 365nm wavelength UV lamp to detect broth fluorescence.

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 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect broth 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 the tubes, approximately 3 to 4 inches (7 to 10cm) away.
3. The presence of *E. coli* in the broth will fluoresce a blue glow.
4. 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.

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 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

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:

		Incubation	

Test Organisms	Inoculation Method*	Time	Temperature	Atmosphere	Results
Escherichia coli ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth; gas bubble in durham tube and fluorescence
Klebsiella aerogenes ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth; gas bubble in durham tube
Staphylococcus aureus ATCC® 25923	B	18-48hr	35°C	Aerobic	Inhibited
Enterococcus faecalis ATCC® 29212	B	18-48hr	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™ Brilliant Green Bile Broth with MUG powder should appear homogeneous, free-flowing, and greenish-beige in color. The prepared media should appear clear, and emerald green in color.

REFERENCES

1. Eaton, Clesceri and Greenberg, (ed.). 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. APHA, Washington, D.C.

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

3. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

4. Vanderzant, C. and D.F. Splittstoesser, (ed.). 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. APHA, Washington, D.C.

5. Marshall, Robert T. 1992. *Standard Methods for the Examination of Dairy Products*, 16th ed. APHA, Washington, D.C.

6. *Appl. Environ. Microbiol.*; 48:285. 1984.

7. *Appl. Environ. Microbiol.*; 43:1320. 1982.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

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

HDQA 2207B [D]