IFU

Instructions for Use

CRITERION™ VIOLET RED BILE AGAR WITH MUG

Cat. no. C7250	CRITERION™ Violet Red Bile Agar with MUG	81.6gm
Cat. no. C7251	CRITERION™ Violet Red Bile Agar with MUG	500gm
Cat. no. C7252	CRITERION™ Violet Red Bile Agar with MUG	2kg
Cat. no. C7253	CRITERION™ Violet Red Bile Agar with MUG	10kg
Cat. no. C7254	CRITERION™ Violet Red Bile Agar with MUG	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Violet Red Bile Agar with MUG is recommended for the detection of *Escherichia coli* and total coliforms in food and dairy products.

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[1-3]

Violet Red Bile Agar is a selective medium used to detect and enumerate lactose-fermenting coliform microorganisms. This media is specified as a standard methods procedure for use in the microbiological analysis of milk and other dairy products. Lactose-fermenting microorganisms produce pink to red colonies that are generally surrounded by a reddish zone of precipitated bile. Non-lactose-fermenting microorganisms result in colorless colonies.

The addition of 4-methylumbelliferyl-beta-D-glucuronide (MUG) allows presumptive identification of *Escherichia coli* from the primary plating medium. Most strains of *E. coli* (96-97%) produce glucuronidase, an enzyme that hydrolyzes MUG to 4-methylumbelliferone. This compound fluoresces under long-wave ultraviolet light (366nm). The addition of MUG to this formulation allows colonies of beta-glucuronidase positive strains of *E. coli* to exhibit blue fluorescent halos when examined under long-wave UV light.

The medium contains bile salts and crystal violet which serve as inhibitory agents toward some gram-positive microorganisms, especially staphylococci. Neutral red is employed as the pH indicator. The peptone serves as a source of carbon, nitrogen, vitamins and minerals. Lactose is also used as a carbohydrate source, while yeast extract provides B vitamins which enhance bacterial growth. Agar is used for solidification.

FORMULA*

Gram weight per liter:	40.8gm/L
Lactose	10.0gm
Gelatin Peptone	7.0gm

Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Bile Salts No. 3	1.5gm
MUG (4-methylumbelliferyl-beta-D-glucuronide)	0.1gm
Neutral Red	0.03gm
Crystal Violet	0.002gm
Agar	15.0gm

Final pH 7.4 +/- 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 reddish-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 40.8gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling to dissolve completely. Do not boil for more than two minutes.

3. Do not autoclave.

4. Cool to 45-50ºC. and aseptically pour plates.

PROCEDURE AND INTERPRETATION OF RESULTS

Examine plates for colonies showing typical colony morphology, color, and fluorescence after incubation. *E. coli* colonies will be pink to red under ambient light. Positive MUG reactions exhibit a blue fluorescence in the colonies under long-wave UV light. Typical strains of *E. coli* exhibit blue fluorescence on VRB 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 366nm or 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.

LIMITATIONS[1-5]

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.

Strains of glucuronidase-negative *E.coli* have been reported as well as glucuronidase-positive strains that do not fluoresce.

If a *Salmonella* or *Shigella* strain is encountered that fluoresces and exhibits colorless growth, differentiate from *E. coli* using other parameters such as gas production, lactose fermentation, growth at 44.5 degrees C. or further biochemical and/or serological tests.

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:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	22-26hr	35°C	Aerobic	Growth; pink-red colonies, red precipitate around colonies and fluoresce (+) under UV light
Salmonella enterica ATCC® 14028	A	22-26hr	35°C	Aerobic	Growth; colorless colonies, fluoresce (-) under UV light
Enterococcus faecalis ATCC® 29212	B	22-26hr	35°C	Aerobic	Partial to complete inhibition

* 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™ Violet Red Bile Agar with MUG powder should appear homogeneous, free-flowing, and reddish-beige in color. The prepared media should appear clear to slightly opalescent, and reddish-purple in color.

REFERENCES

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

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

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10289[C]

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:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207B [D]