IFU

Instructions for Use

CRITERION™ LB AGAR, MILLER

Cat. no. C9280	CRITERION™ LB Agar, Miller	80.0g
Cat. no. C9281	CRITERION™ LB Agar, Miller	500g
Cat. no. C9282	CRITERION™ LB Agar, Miller	2kg
Cat. no. C9283	CRITERION™ LB Agar, Miller	10kg
Cat. no. C9284	CRITERION™ LB Agar, Miller	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ LB Agar, Miller is recommended for the cultivation and maintenance of *Escherichia coli* used in molecular biology procedures.

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

SUMMARY

LB, or "lysogeny broth," media formulations have been widely used for the cultivation of *Escherichia coli* since the 1950s, and have become an industry standard in molecular microbiology applications for the preparation of plasmid DNA and the growth of recombinant strains.[3,5-8] LB medium was originally formulated by Giuseppe Bertani and published in 1951 and has since been modified by Miller, Lennox, and Luria: the formulations differ in the concentration of sodium chloride, which provides for greater selectivity.[3] LB, Miller contains 10gm of sodium chloride; LB, Lennox contains 5gm of sodium chloride; and Luria, Miller contains 0.5gm of sodium chloride.[3,5-8] Low salt formulations, such as those designed by Lennox and Luria, are ideal for salt-sensitive applications.

Adapted by J.H. Miller, LB Agar, Miller is a nutritionally rich medium designed for the growth and culture of pure recombinant strains used in genomic testing.[8] Hardy Diagnostics CRITERION™ LB Agar, Miller is based on the original recipe by Miller and contains casein peptone and yeast extract for amino acids, vitamins, and essential minerals. Sodium chloride provides sodium ions for transport and helps maintain osmotic balance. Agar is added as the solidifying agent.

Additional selective agents, such as ampicillin, carbenicillin, chloramphenicol, streptomycin, tetracycline, or kanamycin, can be added when preparing the medium and are ideal for selective applications; sucrose or glucose can also be added to provide an additional level of selection. The chromogen X-GAL (5-bromo-4-chloro-3-indolyl-galactopyranoside) and supplement IPTG (isopropyl beta-D-thiogalactopyranoside) can be utilized to distinguish *lacZ* transformed cells, making it easy to differentiate between *lac*+ (blue) and *lac*- (white) colonies, if desired.

FORMULA*

Gram weight per liter:	40.0g/L

Tryptone	10.0g
Sodium Chloride	10.0g
Yeast Extract	5.0g
Agar	15.0g

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) that contain dehydrated culture medium at 2-30ºC and do not remove the container desiccant, if applicable. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original light tan.

Store the prepared culture media in sealed containers at 2-8ºC away from light.

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

2. Heat to boiling to dissolve completely.

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

4. Cool to 45-50ºC and dispense as desired.

Note: The shelf life of in-house prepared media from dehydrated culture media is dependent upon preparation methods, container quality, equipment, storage conditions, and batch testing criteria and must be validated by the end user. Refer to *USP Microbiological Best Laboratory Practices <1117>* for more information on validation procedures.[1]

PROCEDURE

For information on procedures and interpretation of results, follow laboratory protocol or consult appropriate references.

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.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, incubators, tubes, bottles, petri dishes, etc., 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	24-48hr	35°C	Aerobic	Growth

* 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™ LB Agar, Miller powder should appear homogeneous, free-flowing, and light tan in color. The prepared medium should appear slightly opalescent and very light amber in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1994. *Current Protocols in Molecular Biology* . Vol. 1. Current Protocols, New York, N.Y.

3. Bertani, G. 1951. Studies on Lysogenesis: The Mode of Phage Liberation by Lysogenic *Escherichia coli* . *J. Bacteriol.* ; 62:293-300.

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

5. Lennox, E.S. 1955. Transduction of Linked Genetic Characteristics of the Host by Bacteriophage P1. *Virology* ; 1:190.

6. Luria, S.E., and J.W. Burrous. 1957. Hybridization Between *Escherichia coli* and *Shigella* . *J. Bacteriol.* ; 74:461-476.

7. Luria, S.E., J.N. Adams, and R.C. Ting. 1960. Transduction of Lactose-Utilizing Ability Among Strain of *E. coli* and *S. dysenteriae* and the Properties of the Transducing Phage Particles. *Virology* ; 12:348-390.

8. Miller, J.H. 1972. *Experiments in Molecular Genetics* . Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

9. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual* , 2nd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.

10. Sambrook and Russell. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.

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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

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

HDQA 2207B [D]