IFU

Instructions for Use

LB AGAR, MILLER POWDER

Cat. no. C6000	CulGenex™ LB Agar, Miller, 2L	80gm
Cat. no. C6001	CulGenex™ LB Agar, Miller	500gm
Cat. no. C6002	CulGenex™ LB Agar, Miller	2kg
Cat. no. C6003	CulGenex™ LB Agar, Miller	10kg
Cat. no. C6009	CulGenex™ LB Agar, Miller, 0.5L	6 pouches/pack

INTENDED USE

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

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

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 Agar, Miller medium contains 10gm of sodium chloride; LB Agar, Lennox contains 5gm of sodium chloride; and Luria Agar, Miller contains 0.5gm of sodium chloride.[3,5-8] Low salt formulations, such as those by Lennox and Luria, are ideal for salt-sensitive applications.

Adapted by J.H. Miller, LB Agar is a nutritionally rich medium designed for the growth and culture of pure recombinant strains used in genomic testing.[8] Hardy Diagnostics CulGenex™ LB Agar media formulations are based on the original recipe by Miller and contain 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 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:	40.0gm/L
Yeast Extract	5.0gm
Tryptone	10.0gm
Sodium Chloride	10.0gm
Agar	15.0gm

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 container(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep container 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 tan.

Store the prepared 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 (20gm per 500ml).

2. Heat to boiling to dissolve completely.

3. Autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC. Add supplements as needed. Dispense into sterile petri dishes.

LIMITATIONS

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclave, incinerators, and incubators, etc., are not provided.

QUALITY CONTROL

The following organism is 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

End users of commercially prepared 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. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

CulGenex™ LB Agar, Miller powder should appear homogeneous, free-flowing, and very light to light tan in color. The prepared media 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.

IFU-10368[A]

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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 2207C [D]