IFU

Instructions for Use

LB AGAR, LENNOX MEDIA

Cat. no. G77	LB Agar, Lennox, 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics CulGenex™ LB Agar, Lennox Media are recommended for the cultivation of recombinant *Escherichia coli* strains used in phage production and in molecular studies.

This product is not intended to be used for the diagnosis of human disease.

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 recombinant strains of *E. coli*.[3,5-8] In general, typical wild type strains of *E. coli* are capable of growth on minimal media. However, researchers have exploited auxotrophic mutations in strains used for molecular applications, making them dependent upon specific growth factors; one strain in particular, *E. coli* K-12, is deficient in vitamin B production and is incapable of growth on nutritionally deficient media.

The original recipe for LB medium was formulated by Giuseppe Bertani and published in 1951.[3] LB media have since been adapted by Miller, Lennox and Luria to contain differing concentrations of sodium chloride in order to provide the appropriate osmotic conditions for the strain of interest. LB Agar, Lennox contains half the sodium chloride concentration of LB Agar, Miller and ten times that found in Luria Agar, Miller.[3,5-8] Low salt formulations, such as those adapted by Lennox and Luria, are ideal for salt-sensitive applications.

LB Agar, Lennox was adapted by E.S. Lennox in the mid 1950s and is a nutritionally rich medium designed to contain certain trace elements for the growth and maintenance of pure recombinant strains. Hardy Diagnostics LB Agar, Lennox Media are based on this formulation and contain tryptone and yeast extract for amino acids, vitamins and essential minerals. The moderate amount of sodium chloride (0.5%) provides sodium ions for transport and helps maintain osmotic balance. Agar is the solidifying agent.

There are many factors critical to the transformation process and, in general, *E. coli* is not naturally transformable. Competency, or the ability to take up extrachromosomal DNA, may be induced by chemical means using divalent cations like calcium or magnesium; divalent cations increase transformation efficiency by increasing cell membrane permeability. Additional agents such as ampicillin, carbenicillin or kanamycin are ideal for selective applications.

FORMULA

Ingredients per liter of deionized water:*

Tryptone	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm
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

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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.

PROCEDURE

Consult listed references for recommended test procedures.[2,5,8-10]

INTERPRETATION OF RESULTS

Growth is evident by the formation of isolated colonies or a confluent lawn of bacteria.

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 loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 8739	J	1-5 days	35°C	Aerobic	Growth
Staphylococcus aureus ATCC® 6538	J	1-5 days	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

LB Agar, Lennox should appear slightly opalescent, and 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-10366[A]

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 2207C [D]