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Instructions for Use

LB AGAR MEDIA

Cat. no. G81	LB Agar with 100ug/ml Ampicillin, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. G194	LB Agar with 50ug/ml Kanamycin, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. G401	LB Agar, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. G407	LB Agar with 100ug/ml Carbenicillin, 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics CulGenex™ LB Agar Media formulations are recommended for the cultivation of bacteria used in molecular biology 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 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 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 the solidifying agent.

Additional agents such as ampicillin, carbenicillin, tetracycline, or kanamycin are ideal for selective applications; sucrose or glucose are added to some formulas to provide an additional level of selection. The chromogen X-GAL (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) used with 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.

FORMULA

Ingredients per liter of deionized water:*

LB Agar (Cat. no. G401):	
Casein Peptone	10.0gm
Sodium Chloride	10.0gm
Yeast Extract	5.0gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC

LB Agar with 100ug/ml Ampicillin (Cat. no. G81) also contains:	
Ampicillin	0.1gm

Final pH 7.0 +/- 0.3 at 25ºC.

LB Agar with 50ug/ml Kanamycin (Cat. no. G194) also contains:	
Kanamycin	0.05gm

Final pH 7.0 +/- 0.3 at 25ºC.

LB Agar with 100ug/ml Carbenicillin (Cat. no. G407) also contains:	
Carbenicillin	0.1gm

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 growth.

Colonies of *lacZ* gene transformed cells grown on media containing X-GAL and IPTG should appear blue in color.

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:

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Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Cat. no. G401:					
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Cat. no. G81 and G194:					
Pseudomonas aeruginosa ATCC® 27853	A	18-24hr	35°C	Aerobic	Growth
Escherichia coli	A	18-24hr	35°C	Aerobic	Partial to complete inhibition

ATCC® 25922					

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Cat. no. G407:					
Klebsiella pneumoniae ATCC® 13883	A	18-24hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition
Pseudomonas aeruginosa ATCC® 27853	B	18-24hr	35°C	Aerobic	Partial to complete inhibition

* 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 (Cat. no. G401), LB Agar with 100ug/ml Ampicillin (Cat. no. G81), and LB Agar with 100ug/ml Carbenicillin (Cat. no. G407) should appear translucent, and light amber in color.

LB Agar with 50ug/ml Kanamycin (Cat. no. G194) should appear clear and slightly opalescent, and light to medium amber in color.

REFERENCES

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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*;

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

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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.

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

HDQA 2207C [D]