LB Agar, Miller • LB Broth, Miller

Intended Use

LB Agar, Miller and LB Broth, Miller (Luria-Bertani) are used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Agar, Miller and LB Broth, Miller are based on LB Medium as described by Miller for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.[1-3] These are nutritionally rich media designed for growth of pure cultures of recombinant strains. *E. coli* grows more rapidly because they provide the cells with amino acids, nucleotide precursors, vitamins and other metabolites that the microorganism would otherwise have to synthesize.[4]

LB Broth, Miller contains twenty times the sodium chloride level of Luria Broth Base, Miller and twice the level found in LB Broth, Lennox.[3,5,6] This allows the researcher to select the optimal salt concentration for a specific strain in LB Agar, Miller.

User Quality Control

Identity Specifications

Difco™ LB Agar, Miller

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Very light amber, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ LB Broth, Miller

Dehydrated Appearance:	Off-white to beige, free-flowing, homogeneous.
Solution:	2.5% solution; soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Very light amber, clear to very slightly opalescent.
Reaction of 2.5% Medium at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ LB Agar, Miller or LB Broth, Miller

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	33526 (K802)	10^2-10^3	Good

Principles of the Procedure

Peptone provides nitrogen and carbon. Vitamins (including B vitamins) and certain trace elements are provided by yeast extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is the solidifying agent in LB Agar, Miller.

Formulae

Difco™ LB Agar, Miller

Approximate Formula* Per Liter

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

Difco™ LB Broth, Miller

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ LB Agar, Miller – 40 g;
   Difco™ LB Broth, Miller – 25 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[3-5]

Expected Results

Growth should be evident on the agar medium by the appearance of colonies and/or a confluent lawn on the surface of the medium. In the broth medium, growth is evident by the appearance of turbidity.

References

1. Luria and Burrous. 1955. J. Bacteriol. *74*:461.
2. Luria, Adams and Ting. 1960. Virology *12*:348.
3. Miller. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol. 1. Greene Publishing Associates, Inc., Brooklyn, N.Y.
5. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. Lennox. 1955. Virology *1*:190.

Availability

Difco™ LB Agar, Miller

Cat. No.	244520	Dehydrated – 500 g
	244510	Dehydrated – 2 kg

Difco™ LB Broth, Miller

Cat. No.	244620	Dehydrated – 500 g
	244610	Dehydrated – 2 kg

LB Agar, Lennox • LB Broth, Lennox

Intended Use

LB Agar, Lennox and LB Broth, Lennox are used for maintaining and cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

LB Agar, Lennox and LB Broth, Lennox are nutritionally rich media developed by Lennox for the growth and maintenance of pure cultures of recombinant strains of *E. coli*.[1] These strains are generally derived from *E. coli* K12, which are deficient in B vitamin production. This strain of *E. coli* has been further modified through specific mutation to create an auxotrophic strain that is not capable of growth on nutritionally deficient media.

LB Agar, Lennox provides all the nutritional requirements of these organisms. LB Agar, Lennox contains half the sodium chloride level of the Miller formulation of LB Agar.[2] This allows the researcher to select the optimal salt concentration for a specific strain.

LB Broth, Lennox contains ten times the sodium chloride level of Luria Broth Base, Miller and one half of that found in LB Broth, Miller.[3] This allows the researcher to select the optimal salt concentration for a specific strain. If desired, the medium may be aseptically supplemented with glucose to prepare the complete medium described by Lennox.

User Quality Control

Identity Specifications

Difco™ LB Agar, Lennox

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent.
Prepared Appearance:	Medium amber, very slightly to slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ LB Broth, Lennox

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Very light amber, clear to very slightly opalescent.
Reaction of 2.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ LB Agar, Lennox or LB Broth, Lennox

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY AGAR	RECOVERY BROTH
Escherichia coli	23724 (C600)	10^2-3×10^2	Good	N/A
Escherichia coli	33694 (HB101)	10^2-3×10^2	Good	Good
Escherichia coli	33849 (DH1)	10^2-3×10^2	Good	N/A
Escherichia coli	39403 (JM103)	10^2-3×10^2	Good	Good
Escherichia coli	47014 (JM107)	10^2-3×10^2	Good	N/A
Escherichia coli	53868 (DH5)	10^2-3×10^2	Good	Good

Principles of the Procedure

Peptone provides nitrogen and carbon. Vitamins (including B vitamins) and certain trace elements are provided by yeast extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is the solidifying agent in LB Agar, Lennox.

Formulae

Difco™ LB Agar, Lennox

Approximate Formula* Per Liter

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

Difco™ LB Broth, Lennox

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ LB Agar, Lennox – 35 g;
   Difco™ LB Broth, Lennox – 20 g.
   Mix thoroughly.
2. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.[1-4]

Expected Results

After sufficient incubation, the agar medium should show growth as evidenced by formation of colonies and/or a confluent lawn of growth. In the broth medium, growth is evident by the appearance of turbidity.

References

1. Lennox. 1955.Virology *1*:190.
2. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current protocols in molecular biology, vol. 1. Green Publishing Associates, Inc., Brooklyn, N.Y.
3. Miller. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Availability

Difco™ LB Agar, Lennox

Cat. No. 240110 Dehydrated – 500 g

Difco™ LB Broth, Lennox

Cat. No. 240230 Dehydrated – 500 g
240210 Dehydrated – 2 kg
240220 Dehydrated – 10 kg