

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.

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.

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% Solution 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
<i>Escherichia coli</i>	33526 (K802)	10 ² -10 ³	Good

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

- Suspend/dissolve the powder in 1 L of purified water:
Difco™ LB Agar, Miller – 40 g;
Difco™ LB Broth, Miller – 25 g.
Mix thoroughly.
- Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- 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

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

Availability

Difco™ LB Agar, Miller

CCAM

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

Difco™ LB Broth, Miller

Cat. No.	244620	Dehydrated – 500 g
	244610	Dehydrated – 2 kg
	214906	Dehydrated – 10 kg

Europe

Cat. No.	257269	Prepared Bottles, 450 mL – Pkg. of 10
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