Intended use
Medium for the cultivation and maintenance of recombinant strains of Escherichia coli for genetic and molecular studies; may be used for routine isolation, cultivation of not particularly fastidious microorganisms.

Presentation
Bottle containing 500 g of media

Storage
Store below 30°C in tightly closed container and the prepared medium at 2-8°C.

Compostion

Casein enzymichydrolysate	10,0	g
Yeast extract	5,0	g
Sodium chloride	10,0	g
Agar	15,0	g

Formula adjusted, standardised to suit performance parameters

Instructions for preparation
Suspend 40 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired.
Mix well and pour into sterile Petri plates.

Principle of the method and general information
General information
LB-Agar (Miller) is prepared as described by Lennox (1) for cultivation and maintenance of recombinant strains of Escherichia coli. The media is nutritionally rich for the growth of pure cultures of recombinant strains. Strains derived from Escherichia coli K12 are deficient in Vitamin B synthesis are further modified by specific mutation to create auxotrophic strains and are therefore unable to grow on nutritionally deficient media. Casein enzymic hydrolysate provides peptides and peptones while Vitamin B complex is provided by yeast extract. Sodium chloride provides sodium ions for membrane transport and also maintain the osmotic equilibrium of the medium.

Instruction for use
Streak a prepared plate of LB agar with a pure or mixed bacterial culture. Incubate inverted plate at recommended temperature of 35-37°C for 24 to 48 hours. Observe colony morphology.
For genetic and molecular studies refer appropriate references for standard procedures (2,3,4)

Limitations
Due to varied nutritional requirement, some strains may grow poorly or fail to grow on this medium.

Quality Control
Physical/Chemical Control
Appearance: Cream to yellow homogeneous free flowing powder

pH: 7,5 ± 0,2 at 25 °C

Gelling: Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium : Yellow to amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction of 4.0% w/v aqueous solution at 25°C.: pH : 7,5 ± 0,2

Microbiological Control

Cultural characteristics observed after an incubation at 35-37°C for 18- 24 hours.
(Inoculum 50-100)

Test Strains		Growth
Escherichia coli	ATCC 23724	Good (Recovery ≥ 70%)
Escherichia coli	ATCC 25922	Good (Recovery ≥ 70%)
Escherichia coli DH5 alpha	MTCC 1652	Good (Recovery ≥ 70%)

Bibliography

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