

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 5000 g of media

Storage

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

Composition

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

<i>Escherichia coli</i>	ATCC 23724	Good (Recovery ≥ 70%)
<i>Escherichia coli</i>	ATCC 25922	Good (Recovery ≥ 70%)
<i>Escherichia coli DH5 alpha</i>	MTCC 1652	Good (Recovery ≥ 70%)

Growth

Bibliography

- Lennox E.S., 1955, Transduction of Linked Genetic Characters of the host by bacteriophage P1., Virology, 1:190.
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- Sambrook J., Fritsch E.F. and Maniatis T., 1989, Molecular Cloning: A Laboratory Manual, 2nd Ed., ColdSpringHarbor Laboratory, Cold Spring Harbor, N.Y.
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