Luria Agar Base, Millers Modification

M1726

Luria Agar Base, Miller's modification is used for the cultivation and maintenance of recombinant strains of *Escherichia coli* with or without addition of glucose.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Sodium Chloride	0.500
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired add 10 ml of 20% glucose solution. Mix well and pour into sterile Petri plates.

Principle And Interpretation

This medium is based on original formula described by Miller for the growth and maintenance of *E.coli* strains used in molecular microbiology (1). Luria Agar Base Miller is a nutritionally rich medium recommended for growth of pure cultures of recombinant strains. *E.coli* is grown in late log phase in LB medium. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol can be added to inhibit host synthesis and as a result prevent replication of the bacterial chromosome. (2)

Luria Agar Base, Miller's modification contains one tenth and one twentieth the sodium chloride level of the Lennox and Miller formulations of LB Agar respectively (1,2,3).This helps the user to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

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 maintains the osmotic equilibrium of the medium. Agar acts as a solidifying agent.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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
Reaction of 3.05% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M1726: Cultural characteristics observed after an incubation of 18 24 hours at 35-37°C with added 1 ml of 20% dextrose solution to 100 ml of M1726.

Organism	Inoculum (CFU)	Growth	Recovery

Cultural Response

Escherichia coli ATCC 23724	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Escherichia coli DH5 alpha MTCC 1652	50-100	luxuriant	>=70%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Miller ,J.H. 1772.Experiments in molecular genetics. Cold spring Harbor Laboratory<(>,<)>Cold spring Harbor, New York.
2. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989.Molecular cloning: A laboratory manual, 2nd ed., Cold Spring Harbor Laboartory, Cold Spring Harbor, New York.
3. Lennox E.S. 1955, Transduction of Linked Genetic Characters of the host by bacteriophage P1., Virology, 1:190.

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