

LB Agar (Miller) (NCM0142)

Intended Use

LB Agar (Miller) is used in molecular genetic studies in a laboratory setting and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

LB Agar (Miller), a nutritionally rich medium designed for growth of pure cultures of recombinant strains, is based on the formulation described by Miller. *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number and do not require selective amplification. Some vectors do not replicate so freely and need to be selectively amplified. Chloramphenicol may be added to inhibit host synthesis and prevent replication of the bacterial chromosome.

LB Agar, Miller contains 10 g/L of sodium chloride, different from the levels in Lennox formulations. This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose or antibiotics.

Typical Formulation

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

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 40 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and aseptically add filter sterilized antibiotic if required.

Test Procedure

Consult appropriate references for recommended test procedures.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is clear with no precipitate, yellow.

Expected Cultural Response: Cultural response on LB Agar (Miller) incubated aerobically at 37 ± 1°C and examined for growth after 21-27 hours.

Microorganism	Approx. Inoculum (CFU)	Recovery
<i>Bacillus subtilis</i> ATCC® 9372	50-200	>70%
<i>Escherichia coli</i> ATCC® 25922	50-200	>70%

The organisms listed are the minimum that should be used for quality control testing.

Technical Specification Sheet



Results

After sufficient incubation the medium should show growth as evidenced by formation of isolate colonies and/or confluent lawn of growth.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New York.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.
3. Lennox E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology. 1:190-206.

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