LB Agar (Miller)

High-Salt Bacterial Culture Medium for Routine Molecular Biology

Catalogue Number: AS-1267

Application	Medium Type	Typical pH	HS Code
E. coli cloning & routine culture	Non-selective solid agar medium	7.0 ± 0.2 at 25 °C	3821.00.00

Overview

LB Agar (Miller) is the most widely used general-purpose solid culture medium for the routine cultivation, cloning, and maintenance of recombinant *Escherichia coli* strains in molecular biology laboratories. The Miller formulation, originally described by J.H. Miller in 1972, contains 10.0 g/L sodium chloride — higher than the Lennox (5.0 g/L) and Bertani (0 g/L) variants — making it the global standard for routine *E. coli* work.

This formulation is fully compatible with common antibiotics, supplements, IPTG/X-gal reporter systems, and a wide range of laboratory strains. It supports rapid bacterial growth, high cell density culture, and reliable plasmid maintenance.

Principle of the Medium

Tryptone (10.0 g/L)	Pancreatic digest of casein providing peptides, amino acids, nitrogen, and carbon for rapid E. coli growth
Yeast Extract (5.0 g/L)	Supplies B-vitamins, trace elements, nucleotides, coenzymes, and growth factors essential for E. coli K-12
Sodium Chloride (10.0 g/L)	High-salt Miller formulation — maintains optimal osmotic strength for standard E. coli strains
Agar (15.0 g/L)	Solidifying agent providing a firm, clear growth surface suitable for colony isolation and selection

Typical Composition (per litre)

Component	CAS Number	Function	Amount
Tryptone (Pancreatic Digest of Casein)	73049-73-7	Nitrogen, carbon, amino acids	10.0 g

Yeast Extract	8013-01-2	Vitamins, growth factors, nucleotides	5.0 g
Sodium Chloride	7647-14-5	Osmotic balance (high-salt Miller)	10.0 g
Agar	9002-18-0	Solidifying agent	15.0 g

Total per litre: 40.0 g | Final pH: 7.0 ± 0.2 at 25 °C

Formulation Comparison — LB Variants

Component	Miller (AS-1267)	Lennox (AS-1269)	Bertani
Tryptone	10.0 g/L	10.0 g/L	10.0 g/L
Yeast Extract	5.0 g/L	5.0 g/L	5.0 g/L
NaCl	10.0 g/L	5.0 g/L	0 g/L
Agar	15.0 g/L	15.0 g/L	15.0 g/L
Total solids	40.0 g/L	35.0 g/L	30.0 g/L
Best for	Standard E. coli — global default	Salt-sensitive strains	Very low osmolarity work

Applications

Molecular Biology & Genetic Engineering

- Routine plating of E. coli strains — DH5α, BL21, TOP10, JM109, XL1-Blue
- Plasmid propagation, transformation, and colony screening
- Antibiotic selection plates (ampicillin, kanamycin, chloramphenicol, tetracycline)
- Blue–white screening when supplemented with X-gal (40 µg/mL) and IPTG (0.1 mM)
- Recombinant protein expression colony selection

Phage & Microbiology Research

- Propagation of plasmid-containing E. coli strains
- General bacteriophage and host strain studies
- Overlay plaque assays when combined with top agar

Teaching & General Laboratory Use

- Standard medium for molecular biology instruction
- Routine microbiology cultivation and streak plate techniques
- Quality control of E. coli competent cell preparations

Preparation Instructions

1. Suspend 40.0 g of dehydrated medium in 1 litre of demineralised or purified water.
2. Heat with frequent agitation until fully dissolved.
3. Verify and adjust pH to 7.0 ± 0.2 if required using 1 M NaOH or 1 M HCl.
4. Sterilise by autoclaving at 121 °C for 15 minutes.
5. Cool to 45–50 °C.
6. Add filter-sterilised antibiotics or supplements aseptically at this stage if required.
7. Dispense 20–25 mL per 90 mm sterile Petri dish.
8. Allow to solidify at room temperature on a level surface before use or storage.

Note: Do not overheat or re-autoclave prepared agar. Add heat-sensitive supplements (X-gal, IPTG, antibiotics) only after cooling to 45–50 °C.

Common Antibiotic Selection Concentrations

Antibiotic	Working Concentration	Stock Concentration	Storage
Ampicillin	100 µg/mL	100 mg/mL in water	−20 °C
Kanamycin	50 µg/mL	50 mg/mL in water	−20 °C
Chloramphenicol	25 µg/mL	25 mg/mL in ethanol	−20 °C
Tetracycline	12.5 µg/mL	12.5 mg/mL in ethanol	−20 °C, protect from light
Gentamicin	10 µg/mL	10 mg/mL in water	−20 °C
Spectinomycin	50 µg/mL	50 mg/mL in water	−20 °C

Note: Filter-sterilise all antibiotic stocks through a 0.22 µm membrane. Add aseptically after cooling to 45–50 °C.

Blue–White Screening Supplements

Supplement	Final Concentration	Preparation	Purpose
X-gal	40 µg/mL	20 mg/mL in DMF or DMSO	Chromogenic substrate for β-galactosidase
IPTG	0.1 mM	100 mM in sterile water	Inducer of lac operon

Spread 40 µL of X-gal stock and 4 µL of IPTG stock onto the agar surface 30 minutes before plating. Blue colonies contain lacZ insert; white colonies are recombinants.

Incubation & Typical Growth

Inoculate by surface spread or pour plate. Incubate aerobically at 37 °C for 12–18 hours. Colonies are visible within 12–16 hours.

Organism	Expected Result	Incubation
E. coli K-12 DH5α	Good growth — white/cream 1–3 mm colonies	37 °C, 12–16 h
E. coli BL21(DE3)	Good growth — suitable for expression screens	37 °C, 12–16 h
E. coli TOP10	Good growth — high-efficiency cloning host	37 °C, 12–16 h
E. coli JM109	Good growth — compatible with M13 phage work	37 °C, 12–16 h
Salmonella typhimurium ATCC 14028	Good growth	37 °C, 18–24 h

Storage & Stability

Dehydrated powder	Store at 15–30 °C in a cool, dry, tightly closed container
Protect from	Moisture, direct light, and extreme temperatures
Prepared plates (no antibiotics)	2–8 °C, inverted, up to 4 weeks
Prepared plates (with antibiotics)	2–8 °C, use within 1–2 weeks
Shelf life (dehydrated)	As per labelled expiry date
Do not use if	Contamination, colour change, or cracking of agar surface is observed

PRODUCT DESCRIPTION

AS-1267 | LB Agar (Miller)

www.ausamics.com.au

Quality & Compliance

Manufactured under controlled conditions for batch-to-batch consistency. Performance tested against ATCC reference organisms prior to release. Compatible with protocols described in Miller (1972) Experiments in Molecular Genetics, Sambrook & Russell (Molecular Cloning), and Current Protocols in Molecular Biology.

Reference

Miller, J.H. (1972). *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, New York.

Customs & Trade Information

HS / AHECC Code	3821.00.00
Description	Prepared culture media for the development or maintenance of microorganisms
Country of Origin	Australia

Disclaimer