

The Liu Laboratory protocol — E.coli
Arts & Sci Washington University in St. Louis

Rich Media: Unless otherwise specified, rich media should be autoclaved for 25 min on the liquid cycle at 15 lb/in² (1.05 kg/ cm²). Antibiotics and nutritional supplements should be added only after the solution has cooled to 50°C or below. A flask containing liquid at 50°C feels hot but can be held continuously in one's bare hands. All give the quantity of ingredients per liter of medium.

LB medium

10 g tryptone
5 g yeast extract
5 g NaCl
1 ml 1 N NaOH

The original recipe for LB medium (variously called Luria Broth, Lenox Broth, and Luria-Bertani medium), does not contain NaOH. There are many different recipes for LB that differ only in the amount of NaOH added. We use this formula in our own work. Even though the pH is adjusted to near 7 with NaOH, this medium is not very highly buffered, and the pH of a culture growing in it drops as it nears saturation. For plates (solid), add 15g agar (don't forget putting a stir bar in the container! for cooling before pouring)

TB (terrific broth)

12 g Bacto tryptone
24 g Bacto yeast extract
4 ml glycerol

Add H₂O to 900 ml and autoclave for 15 min at 15 lb/in² (1.05 kg/ cm²) on the liquid cycle, then add to above sterile solution 100 ml of a sterile solution of 0.17 M KH₂PO₄ and 0.72 M K₂HPO₄. In our lab, check TB premixed powder first and follow the protocol.

Minimal Media (for isotope labeling)

M9 medium, 5×

30 g Na₂HPO₄
15 g KH₂PO₄
5 g NH₄Cl
2.5 g NaCl
15 mg CaCl₂ (optional)

As noted below, M9 lacks iron (see below). It can be supplemented with 2.5 mg FeSO₄·7H₂O per liter.

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M63 medium, 5×

10 g $(\text{NH}_4)_2\text{SO}_4$

68 g KH_2PO_4

2.5 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

Adjust to pH 7 with KOH

A medium, 5×

5 g $(\text{NH}_4)_2\text{SO}_4$

22.5 g KH_2PO_4

52.5 g K_2HPO_4

2.5 g sodium citrate $\cdot 2\text{H}_2\text{O}$

Before they are used, concentrated media should be diluted to 1× with sterile water and supplemented with the following sterile solutions, per liter.

1 ml 1 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

10 ml 20% carbon source (sugar or glycerol)

and, if required:

0.1 ml 0.5% vitamin B1 (thiamine)

5 ml 20% Casamino Acids *or*

1 amino acids to 40 $\mu\text{g}/\text{ml}$ *or* 1 amino acids to 80 $\mu\text{g}/\text{ml}$