

Protocol: Luria Broth (LB)

Adapted from Cold Spring Harbor Protocols (<http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8141.full>)

LB Medium is a nutrient-rich medium for the growth of bacteria, especially *E. coli* that is used in molecular biology. The following protocol is for making both LB liquid medium, as well as LB agar.

Reagents

Bacto agar (Becton, Dickinson and Company) (if preparing agar plates)

Bacto tryptone (Becton, Dickinson and Company)

NaCl

Yeast extract

diH₂O

5M NaOH

Note: When autoclaving liquid, the rule of thumb is to make sure the final volume of your liquid is no more than ½ the max volume of the container (e.g., no more than 500 mL of liquid in a 1 L Pyrex bottle.)

1. To an autoclavable bottle, add:

Reagent	Amount to add per 500ml final volume		Amount to add per 250ml final volume	
	Liquid	Agar Plates	Liquid	Agar Plates
Bacto agar	--	7.5 g	--	3.75 g
Bacto tryptone	5 g	5 g	2.5 g	2.5 g
NaCl	5 g	5 g	2.5 g	2.5 g
Yeast extract	2.5 g	2.5 g	1.25 g	1.25 g
diH ₂ O	500ml	500ml	250ml	250ml
5M NaOH	100µL	100µL	50µL	50µL

- a. Weigh out and add dry reagents to autoclavable Pyrex bottle.
 - b. Using a graduated cylinder, measure out the appropriate volume of diH₂O.
 - c. Add diH₂O to the dry reagents and swirl.
 - d. **LASTLY**, add the indicated volume of 5M NaOH.
2. Autoclave the mixture (**You MUST get trained on how to use the autoclave before doing this!**)
 - a. Make sure the screw-caps are **LOOSLEY** screwed on, allowing gas to escape the bottle.
 - b. Place a piece of autoclave tape across the cap, making sure one end is taped to the glass bottle.
 - c. Autoclave for 25 minutes (use "SLOW EXHAUST" setting for liquids).
 3. *For LB liquid media:*

Simply allow the medium to cool completely to room temperature before tightening the lid completely for storage.

For LB agar plates:

- a. Place the bottle in a 55°C in a water bath to allow the temperature of the media to come down. Antibiotic can be added once the medium has cooled to 55°C.
- b. While still warm, pipette 25ml of media into 10cm diameter plastic petri dishes using a serological pipette.
- c. Allow the plates to cool at room temperature.