

# LB Medium Preparation

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## Introduction

In order for bacteria to be successfully cultured, they must be grown in the appropriate media. LB, also known as Lysogeny broth, is a nutrient rich broth that is a standard for culturing *Escherichia coli*, as it allows for quick growth and high yields. Therefore, the proper preparation of LB will be crucial to maintaining our bacterial stock throughout the summer. Furthermore, addition of agar to LB broth creates a gel for bacteria to grow upon, and is therefore used for plating bacterial cultures on petri dishes.

## Materials

- › 10g Bacto-tryptone
- › 2.5g yeast extract
- › 5g NaCl
- › 7.5g agar (Only necessary if making LB agar plates)
- › 500mL of dH<sub>2</sub>O (distilled water)

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## Procedure

### Making the LB broth

1. Obtain a clean 1L pyrex bottle
2. Obtain a graduated cylinder with 500mL of dH<sub>2</sub>O and add to the bottle. Record the amount added.
3. Using filter paper, separately measure out 5g of NaCl, 5g of Tryptone, and 2.5g of yeast extract on a scale and add them to the bottle. Swirl the bottle in a circular motion to mix. Remember to re-calibrate your scales in between measurements.
4. If you are making LB agar plates, weigh and add 7.5g of agar and swirl to mix. Record the amount added.

### Autoclaving

5. Lightly seal the top of the beaker with aluminium foil, and label the beaker with autoclave tape stating LB (agar)–[your name]–[date]–[media number]–iGEM.
6. Use appropriate transportation protocols to bring the LB bottle into the autoclave room. Remember to store the beaker in an autoclavable basin, in case of spills.
7. Check the water level on the autoclave, if necessary. Autoclave on the liquid setting for approximately 20 min.
8. The contents of the beaker will be hot after autoclaving, therefore take the necessary measures to prevent burns.

9. After autoclaving, allow the LB media to cool to 55°C before handling.

Use laser thermometer to check the temperature of the glass.

10. The LB broth can be stored in sterile conditions at room temperature and should be good for 3-4 months. Flame the lip of the bottle each time the LB is used. If the LB contains antibiotics, store in a -4°C freezer.

However, it is not recommended to store LB with antibiotics as the antibiotics will degrade over time

## Pouring the plates

11. Sterilize the workspace with 70% EtOH before depositing your materials. Light the Bunsen burner.

12. Obtain a stack/roll of empty plates. The plates should still be in their plastic sleeve/wrapping, as they should be sterile. Don't throw out the wrapping as it can be used to store the plates. It is essential that you minimize any chance of contaminating the plates. Make sure that you open the package at the top and expose the plates as minimally as possible.

13. Once you take the plates out, store them upside down on your lab bench. Label the plates with [your name]-iGEM 2017-[date]-[media number]-[antibiotic]. Once labelled, you may stack the plates to free up workspace.

14. Allow the LB media to cool before pouring. The LB will start to settle at ~30°C.

15. If you are preparing selective media, add antibiotic to the mixture. Swirl the flask in a circular motion to mix. If you don't know whether or not you are preparing selective media, ASK.

Use concentrated liquid stocks for the antibiotics.

16. Recommended antibiotic concentrations:

Chloramphenicol (CAM): 25 µg/mL

Ampicillin (AMP): 100 µg/mL

17. Take an empty plate and open it slightly. You do not need to open it all the way to pour the agar.

18. Pour agar until 2/3 of the plate has been covered, or approximately half of the plate has been filled when viewed from the side. Pour the agar slowly to prevent the formation of bubbles. Swirl the plate in a circular motion to distribute the media evenly on the plate.

19. If you pour too much LB, you will not be able to produce 25 plates. If you don't pour enough media, it may minimize bacterial growth.

20. After pouring, set the plates to cool in stacks of 4-5 to save space and flip the plates to prevent condensation forming on the agar. Don't stack plates too high - we want to minimize the risk of spills. Allow the plates to cool for at least 20 minutes until the agar has solidified.

21. Rinse the Pyrex bottle with water before the remnants solidify and become hard to remove.

22. The plates can then be stacked and stored in plastic bags (ideally, reuse the plastic bags that the plates came in.)

23. Store LB agar plates in a 4°C freezer. They should be good for 1-2 months.