

How to Make LB Agar Plates

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Proper handling and preparation are crucial when preparing plates for culture.

Agar is a gelatinous substance, manufactured from red algae and used for a variety of purposes. One of its common uses is as a culturing medium for bacteria. LB agar is nutrient agar that has been combined with lysogeny broth (LB). It is a commonly held fallacy that LB stands for Luria-Bertani. However, its inventor, Giuseppe Bertani, states in his paper "Lysogeny at Mid-Twentieth Century: P1, P2, and Other Experimental Systems," that the initials were always intended to stand for lysogeny broth, not his name and that of his coresearcher, Salvador Luria. LB agar is versatile and fairly simple to make.

Difficulty: Moderately Challenging

Instructions

Things You'll Need:

- For 1 L of finished medium:
- Scale and weighing papers or trays
- 1-L graduated cylinder
- 1 L distilled or deionized water
- 2-L Erlenmeyer flask
- 10 g tryptone
- 5 g yeast extract
- 5 g noniodized table salt
- 15 g agar
- Aluminum foil
- Waterproof oven mitts
- Autoclave or pressure cooker
- Sterile Petri plates

1. 1



Use an Erlenmeyer flask that holds twice the volume of the medium you intend to prepare.

Place the tryptone, yeast extract and salt into the Erlenmeyer flask.

2. 2

Add the distilled or deionized water and stir or shake to dissolve most of the dry ingredients.

3. 3

Add the agar and continue to shake or stir. All of the agar won't dissolve, but it's important that you get most of it into solution. Avoid large clumps of undissolved agar settling in the bottom of your flask.

4. 4

Seal the opening of the flask with foil.

5. 5

Place the flask in an autoclave-safe container.

6. 6

Pour about 1 cm of water into the container.

7. 7

Put the container and flask into the autoclave. Set the autoclave for a liquid cycle and sterilize for at least 20 minutes. If you are using a pressure cooker for sterilization, go to Step 8. Otherwise, skip to Step 10.

8. 8

If you are using a pressure cooker, place a small amount of water in the cooker and place the flask in the water. Do not attempt to sterilize more than 250 mL at a time using this method.

9. 9

Bring the pressure cooker to about 20 psi and sterilize for about 30 minutes.

10. 10

Remove the flask from the autoclave or pressure cooker, taking care as the flask will be quite hot.

11. 11

Swirl the flask gently once sterilization is completed. This will distribute the agar more evenly throughout your solution.

12. 12

Put the flask on a suitable surface and allow it to cool a bit. If you have prepared 1 L or more of medium, give it a gentle swirl every 10 to 20 minutes. You will be able to tell that the medium has cooled sufficiently when you can hold the back of your fingers against the flask for about 2 seconds without its being uncomfortably hot to the touch. Wait about 15 to 20 minutes before attempting this.

13. 13

Place a Petri dish on a flat surface. Gently lift the lid at an angle, so that most of the dish is left covered. Do not lift the lid straight off the plate.

14. 14

Pour the medium from the flask into the Petri dish until the dish is about half full.

15. 15

Immediately replace the lid, move the plate aside and move on to the next until all desired plates have been filled. The plates will complete their solidification in about 30 minutes.

16. 16

Place the Petri dishes in the sterile bag in which they arrived or another suitable, sterile container if you are not inoculating them right away. Seal the container to preserve

moisture. Label the plates if desired. If you are storing the plates, they should be kept at a temperature of about 39 to 40 degrees F.

Read more: [How to Make LB Agar Plates | eHow.com](http://www.ehow.com/how_7589312_make-lb-agar-plates.html#ixzz17yIEZKmM)
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