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Making LB Agar Plate

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1 Works for me dx.doi.org/10.17504/protocols.io.3iqgkdw

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ABSTRACT

How to make LB Agar Broth plates for bacterial quantification.

*Protocol image used is from Wikimedia commons [https://commons.wikimedia.org/wiki/File:LB_agar_plate.jpg]

GUIDELINES

Wear gloves.

MATERIALS TEXT

- Deionized water (DIW)
- Sterile petri dishes
- Aluminum foil
- Glassware with a volume at least twice that of the volume of broth to be made

If using premixed Miller LB Agar broth powder, use the recommended ratio. Alternatively, you can use:

- 50 g tryptone
- 2.5g yeast extract
- 5.0 g NaCl
- 7.5 g agar

Mix Broth

1 Add **2.5 g** Miller's LB Agar Powder and **1.5 g** agarose to **100 ml** Deionized Water.

2 Swirl powder in DIW until dissolved.

Sterilize Broth

3 Cover top of glassware containing broth solution with aluminum foil, and add a small piece of thermal indicator tape.

4 Autoclave at **121 °C** for **00:20:00** .

Do not use the "Exhaust" mode on the autoclave as decreasing the pressure so suddenly will result in rapid boiling of solution. Allow the pressure to drop down on its own after the cycle is complete.

Pour Plates

5 Remove broth from autoclave while still hot.

6 With one hand, move petri dish lid just enough to pour the broth.

7 Pour broth into dish so that there is 5-7.5 mm of broth at the bottom.

Store Plates

8 Wrap edge of plate with parafilm to protect from contamination.

9 Once broth is firm enough, invert so condensation does not drip onto the medium.

10 Store plates in a plastic bag at **4 °C** if they will not be used immediately.