OCT 03, 2019

🌐 Making LB Agar Plate

Kenneth Schackart¹

¹University of Arizona

Yoon Lab

Kenneth Schackart
University of Arizona

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3iqgkdw

Protocol Citation: Kenneth Schackart 2019. Making LB Agar Plate. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.3iqgkdw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: May 30, 2019

Last Modified: Oct 03, 2019

PROTOCOL integer ID:
23856

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

- 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 .

Note

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.