

Jun 13, 2019

LB Media

DOI

dx.doi.org/10.17504/protocols.io.3idgka6

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DOI: <https://dx.doi.org/10.17504/protocols.io.3idgka6>

Protocol Citation: William Brydon, Sricharan Kadimi 2019. LB Media. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.3idgka6>

Manuscript citation:

Taken from Benchling Protocol

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Protocol status: Working

We use this protocol in our group and it is working, though open to further improvement/modification.

Created: May 30, 2019

Last Modified: June 13, 2019

Protocol Integer ID: 23845

Keywords: bacterial culture, lb media lb, overnight culture, overnight cultures after transformation, media, luria, culture

Abstract

LB (Luria-Bertani) media is commonly used for bacterial culture. We use it for overnight cultures after transformations.

Materials

MATERIALS

 Yeast Extract **Catalog #Y1625**

 Sodium Chloride **Catalog #PubChem CID: 5234**

 Tryptone **Fisher Scientific Catalog #BP1421-500**

- 1 Liter Glass Bottle
- Autoclave
- Stirring plate and magnetic stirrer

Troubleshooting

Before start

This protocol creates 1L of media.







Creating the Broth

- 1 Put a stir bar into a 1L glass bottle and fill the glass bottle with 1L of DI water (using a graduated cylinder). Mark the level of the water with a thin Sharpie or label tape. Pour out ~50mL of the water. 2m

Note

We do this step because QSing the media in a graduated cylinder gets messy - it's often difficult to transfer the solute back and forth.

- 2 In a large glass bottle (at least 1L), add the following: 10m

<i>Component</i>	<i>Amount</i>
Deionized Water	 950 mL
Tryptone	 10 g
Yeast Extract	 5 g
NaCl	 10 g

(You do not have to add the exact amount of water, just make sure it is close to 950mL).

- 3 Shake or stir to dissolve all the solutes. 20m

- 4 Optional: We have never done this step, but it is recommended by the Sambrook Molecular Cloning manual. 10m

Adjust the pH of the solution to 7.0 using 5M NaOH (~0.2mL).

- 5 QS the solution to the mark you made in Step 1 (1L) using DI water. 1m

Sterlizing the Broth

- 6 Autoclave bottle following Autoclave protocol on the liquid cycle. Choose the appropriate cycle for the amount of liquid you have. Make sure the bottle cap is on loosely 1h

- 7 Let bottle cool on the lab bench with loose cap 30m



- 8 Keep bottle in the cold room for storage, with the cap tightly closed. Make sure you keep the bottle STERILE. We want a clear solution and nothing growing in our broth