

LB (Luria-Bertani) agar

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Abstract

This protocol describes how to make 1 L of Luria-Bertani agar (also known as LB agar) for the culturing of bacteria on plates.

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Guidelines

1 L of LB agar makes ~20 plates.

At 4 °C unused plates can be stored for around 2 weeks prior to use.

Once used, LB agar plates containing bacterial colonies can be stored in air-tight container/bag at 4 °C for a maximum of 2 weeks.

Prior to disposal, plates containing bacterial colonies must be wrapped in Parafilm and autoclaved.

Materials

Agar ash 2.0-4.5% [A7002](#) by [Sigma Aldrich](#)

LB broth, Miller [BP1426-500](#) by [Thermo Fisher Scientific](#)

LB agar, Miller [BP1425-500](#) by [Thermo Fisher Scientific](#)

Protocol

Step 1.

Add 25g LB broth, Miller and 15g agar (or 40g LB agar, Miller) per litre of water.

Step 2.

Mix well by inverting bottle several times until powder dissolved.

Step 3.

Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle.

Step 4.

Following autoclave (while media still liquid but cool enough to safely handle bottle) pour LB agar

from bottle into plates within a laminar flow hood. Allow 50 mL per plate.

NOTES

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The LB agar bottle must only be opened and the plates poured in a laminar flow hood to prevent contamination.

Step 5.

Leave plates in laminar hood until agar sets (30 mins).

Step 6.

When agar has set, replace lid, invert plates and store in an air tight bag at 4 °C.

Step 7.

Plates should be removed from 4 °C at least 1 h before use and placed in 30 °C incubator. To prevent condensation from lid dropping onto agar surface, wipe lids dry with Kimwipe and store plates inverted.

NOTES

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Reminder: only remove lid from LB agar plate while in a laminar flow hood to prevent contamination.

Warnings

Read appropriate SDS for LB broth, Miller and/or LB agar, Miller before use.

Bottle can be extremely hot immediately following autoclave. Use thermal gloves for handling.