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LB (Luria-Bertani) agar

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Protocol status: Working

We use this protocol and it's working

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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.

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

MATERIALS

⊗ Agar ash 2.0-4.5% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7002**

⊗ LB broth, Miller **Thermo Fisher Scientific Catalog #BP1426-500**

⊗ LB agar, Miller **Thermo Fisher Scientific Catalog #BP1425-500**

Safety 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.



- 1 Add 25g LB broth, Miller and 15g agar (or 40g LB agar, Miller) per litre of water.
- 2 Mix well by inverting bottle several times until powder dissolved.
- 3 Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle.
- 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.

Note

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

- 5 Leave plates in laminar hood until agar sets (~30 mins).
- 6 When agar has set, replace lid, invert plates and store in an air tight bag at 4 °C.
- 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.

Note

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