LB agar plate, 1.5% Version 2

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Abstract

Preparation of both LB broth for growing bacteria and LB agar plates

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Protocol

Prepare LB agar

Step 1.

Pour 1.5% w/v (7.5g/500mL) bacteriological grade agar into an autoclave safe container capable of holding at least 500mL



8 g Additional info:



REAGENTS

Agar, bacteriological grade J637 by Amresco

Prepare LB agar

Step 2.

q.s. to 500mL using LB media



500 ml Additional info:



REAGENTS

Luria-Bertani (LB) broth, makes 1L K488 by Amresco

Prepare LB agar

Step 3.

Stir vigorously at room temperature until particulates are no longer visible

Prepare LB agar

Step 4.

Loosely cap bottle and then autoclave for a minimum of 30 minutes (increase to 45 minutes if making 1L) using slow exhaust (liquid) cycle

O DURATION

00:30:00

Prepare LB agar

Step 5.

Remove from autoclave and let cool for 20-40 minutes or until sufficiently cool to permit handling with simple nitrile/latex gloves or bare handed (about 40-60C). [Optional]: Cool in 55C water bath to avoid having the agar cool too much prior to pouring.

© DURATION

00:20:00

Prepare LB agar

Step 6.

Add antibiotic selection after agar has cooled down and mix well

Step 7.

Carefully pour about 20-25mL of LB agar into 10cm Petri dishes (about 20-25 plates)



REAGENTS

Petri dish, 10cm, polystyrene FB0875712 by Fisher Scientific

Step 8.

Let agar cool before placing lid on top and inverting plate. Alternatively, place lid open slightly to the side to allow for evaporation as the agar cools.

Step 9.

Wrap plates in plastic wrap or store sealed in plastic bag to prevent plates from drying out. Wrap in aluminum foil to prevent degradation of light-sensitive antibiotics, e.g. ampicillin. Store plates at 4C, inverted.