

Preparation of Luria Broth/Kanamycin Media

Introduction

This protocol yields 1 L of Luria Broth/Kanamycin growth media for agar plates. **If you wish to create liquid culture media, omit addition of the agar in Step 1 and omit Steps 6-10.**

Reagents

-  Milli-Q H₂O
-  15 g powdered agar
-  10 g sodium chloride
-  10 g tryptone
-  5 g yeast extract
-  50 mg Kanamycin
-  NaOH solution
-  70% ethanol solution

Equipment

-  2 L Erlenmeyer flask
-  Analytical balance
-  Thermometer
-  Aluminum foil
-  Autoclave
-  100 mm x 15 mm petri dishes
-  Human friend

Procedure

1. Measure out the indicated reagents. Place the agar, sodium chloride, tryptone, and yeast extract in a 2 L Erlenmeyer flask. Add 950 mL of Milli-Q H₂O to the flask and shake vigorously.
2. Prepare the pH meter by calibrating using pH buffer solutions.
3. Adjust the pH of the solution using NaOH until the pH is 7.0. Backfill to 1000 mL with Milli-Q H₂O.
4. Cover the mouth of the flask with aluminum foil, taking care not to contaminate the contents.
5. Autoclave the solution for 20 minutes. **The next three steps must be performed rapidly to avoid solidification of the agar before it has been plated.**
6. Using a thermometer to monitor the temperature of the media, allow it to cool to 55°C.
 - a. *If you do not have access to a thermometer, a good indication of an approximate temperature is if you can hold the flask for approximately 10 seconds without injury. Please be careful, we're not responsible for any burns incurred.*
7. Add 50 mg of Kanamycin to the media and mix.
8. Pour approximately 15-25 mL of the media into a 100 mm x 15 mm petri dish.
 - a. *In our experience, it helps have a friend lay the petri dishes out on a large work surface with the edges close to one another while you wait for the media to cool and have them open and close the petri dishes as you pour to prevent contamination. Keep 'em bois warm! Stack the plates on one another after the media has been poured to prevent condensation.*

Preparation of Luria Broth/Kanamycin Media Cont.

9. Let the plates set for approximately 30 minutes at room temperature before storing in a 4°C refrigerator for up to one month.
10. Test the antibiotic by plating an antibiotic resistant bacterial strain in one plate and a non-antibiotic resistant bacterial strain on another, incubate overnight, and compare growth.