

Chloramphenicol LB Broth

Introduction

In order to grow cells in liquid broth, a broth containing the necessary nutrients and an appropriate antibiotic must be used to grow the cells. This solution must be sterilized and stored in the cold room at 4 degrees.

Materials

- › Tryptone
- › Yeast Extract
- › Sodium Chloride
- › dH2O
- › 1 Liter Glass Bottle
- › Autoclave
- › Stock solution chloramphenicol (25 mg/ml)
- › Stirring plate and magnetic stirrer
- ›

Procedure

Creating the Broth Solution

1. In a large glass bottle (at least 1 L), add the following **for a 500 mL solution**:

	A	B
1	Amount	Component
2	5 grams	Tryptone
3	2.5 grams	Yeast Extract
4	5 grams	NaCl
5	Q/S to 500 mL	dH2O

2. Shake until solutes completely dissolve
3. Place magnetic stirring bar inside the bottle
4. Stir (7/10) with heat (about 4/10) and wait about 15-20 minutes for the salt to dissolve. When the bottle is tilted, salt should not collect at the bottom.

A little salt at the bottom is usually fine. This will dissolve once the solution is autoclaved.

Sterilizing the Broth

5. Pour water into a beige plastic bucket to about 1 inch deep
6. Place bottle in bucket
7. Place bucket containing bottle into autoclave and run Liquids cycle, quickest one for small amount of liquid.
8. Once done, use hot hands to take bottle out of autoclave (be very careful) and place on a stirring plate
9. Once the temperature in the bottle reaches 55°C
10. Continue stirring and keep labeled and dated solution in cold room.
11. Add liquid chloramphenicol to individual tubes as needed in a 5 ul to 5 mL for a final concentration of 35 ug/mL