Antibacterial Stock Preparation

Introduction

The preparation of antibiotic stock is a relatively simple series of mixing and dilutions. The antibiotic stocks are typically made from four antibiotics: Ampicillin, Kanamycin, Tetracycline and Chloramphenicol. With the exception of chloramphenicol, the other antibiotics are light sensitive and once stock is prepared they are wrapped in foil to reduce light exposure.

An antibiotic comes in a powder form as the basic stock and is mixed with milli-Q water to form a stock solution. Antibiotic stock preparation follows the same basic process for each antibiotic, varying only in concentration which is dependent on the type of antibiotic used.

Antibiotic stocks are prepared for use either in liquid or solid media. In the case of liquid media, the antibiotic stock is simply added and mixed in. For solid media, usually agar and LB, the media is sent for autoclaving, and while the media is still hot the antibiotic is added and mixed. This is to ensure that the antibiotic can be mixed evenly amongst the media before it solidifies into a solid state.

Antibiotics are used for the purpose of selection. In terms of synthetic biology, plasmids confer selective antibiotic resistance when successfully transformed into their target bacteria. Using liquid or solid growth media that has been treated with antibiotics provides a selection control for those bacteria that have successfully incorporated the plasmid.

Materials

Reagents

- MilliQ water
- 4 g Ampicillin
- 800 mg Kanamycin
- 400 mg Tetracycline
- 2.72 g Chloramphenicol
- 80 mL 70% EtOH
- 80 mL 100% EtOH

Equipment

- Antistatic weighing boat
- 100 mL Pyrex bottle
- Magnetic stir bar
- Magnetic stirrer
- Analytical balance
- Milli-Q water dispenser
- 50 mL Falcon tubes

- Aluminum foil
- 20 mL syringe
- 0.22 µm Filter
- 1 mL microcentrifuge tubes

Ampicillin

Notes

Stocks and Usage:

- Stock concentration 50mg/mL in milliQ water
- Aliquots: 500µL (use a P1000 set to 0500)
- Working Concentration: 50µg/mL preparation of 80 mL stock solution

Ampicillin is kept in the 4°C fridge, and is light sensitive. To ensure your stock solution is not degraded, cover all microcentrifuge tubes used for storing the solution with foil (you could use a falcon tube and wrap it with foil as well).

Procedure

1. Weigh 4g ampicillin onto an antistatic weighing boat.
2. Add 80mL milliQ water to a 100 mL Pyrex bottle.
3. Add the ampicillin to the milliQ water.
4. Place a small magnetic stir bar into the solution and place the Pyrex bottle on the stirrer. Set at 300-600 rpm and stir until dissolved.
5. Filter sterilise the solution into 50 mL Falcon tubes using a 20mL syringe outfitted with a 0.22µm filter.
6. Aliquot into the appropriate microcentrifuge tubes, labelled with an "A" on top, and store in the Nalgene racks found in the 20°C fridge

Kanamycin (KAN)

Notes

Stocks and Usage:

- Stock Concentration 10mg/mL in milliQ water
- Aliquots: 1mL (use a P1000 set to 1000)
- Working Concentration: 50µg/mL preparation of 80 mL stock solution
- Add 5 mL of stock per litre of LB

Kanamycin is kept in the 4°C fridge, and is light sensitive. To ensure your stock solution is not degraded, cover all microcentrifuge tubes used for storing the solution with foil.

Procedure

1. Weigh 800 mg kanamycin onto an antistatic weighing boat.
2. Add 80mL milliQ water to a 100 mL Pyrex bottle.

3. Add the kanamycin to the milliQ.
4. Place a small magnetic stir bar into the solution and place the Pyrex bottle on the stirrer. Set at 300-600 rpm and stir until dissolved.
5. Filter sterilise the solution into 50 mL Falcon tubes using a 20mL syringe outfitted with a 0.22µm filter
6. Aliquot (1mL) into the appropriate microcentrifuge tubes, labelled with a "K" on top. Store in the Nalgene racks found in the 20°C fridge. (Make sure microcentrifuge tubes are covered with foil)

Chloramphenicol (CAM)

Notes

Stocks and Usage:

- Stock Concentration 25mg/mL in 100% EtOH (N.B. 100%, not 70%!)
- Aliquots: 1mL (P1000 set to 1000)
- Working Concentration: 34µg/mL preparation of 80 mL stock solution

Chloramphenicol is kept with the general chemicals, and is not light sensitive. The microcentrifuge tubes do not need to be covered with foil to store chloramphenicol.

Procedure

1. Weigh 2.72g chloramphenicol onto an antistatic weighing boat.
2. Add 80mL 100% EtOH to a 100 mL Pyrex bottle.
3. Add the chloramphenicol to the 100% EtOH.
4. Place a small magnetic stir bar into the solution and place the Pyrex bottle on the stirrer. Set at 300-600 rpm and stir until dissolved.
5. Aliquot (1mL) into the appropriate microcentrifuge tubes, labelled with a "C" on top. Store in the Nalgene racks found in the 20°C fridge.

Note: No filter sterilization is needed because it is stored in 100% EtOH.

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Protocols of previous iGEM teams were used to make this guideline.