

STANDARD OPERATING PROCEDURE

Title: Preparation of LB media and LB plates +/- Antibiotic

SOP#: R-104

Version #: 1

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1. PURPOSE

This procedure is to be used for the preparation of LB media and LB plates +/- antibiotic.

2. SCOPE

This document describes the procedure for the preparation of LB media and plates with and without antibiotic.

3. RESPONSIBILITIES

It is the responsibility of the person(s) preparing these reagents to be familiar with lab safety procedures and to have basic laboratory skills.

4. EQUIPMENT

- Getinge 533LS Autoclave
- Stir Plate Thermolyne SAFE-T SHP9
- 0.22µm filter unit (Fisher Scientific, Cat. # 09-761-118)

5. MATERIALS

- Premixed (LB) Luria Broth (Fisher Scientific, Cat. # 50842699)
- BactoAgar (Invitrogen, Cat. # 15510-027)
- Ampicillan (Fisher Scientific, Cat. # BP1760-25)
- Kanamycin (Sigma Aldrich, Cat. # K-1377)
- Chloramphenicol (Sigma Aldrich, Cat. # C1919-5G)
- Ethanol (Sigma Aldrich, Cat. # E7023)
- Petri Dishes (VWR Scientific, Cat. # 25384-342)

6. REAGENTS

N/A

7. PROCEDURE

7.1. **LB Media +/- Antibiotic**

- 7.1.1. Add 15.5 grams of premixed LB to 1L of dH₂O
- 7.1.2. Autoclave for 30 minutes
- 7.1.3. Cool the media till ~50°C (this is when you can pick up the flask without a glove)
- 7.1.4. If desired add 1 ml of antibiotic (ampicillan (150 mg/ml), kanamycin (30 mg/ml) or chloramphenicol (34 mg/ml)) to the cooled 1L LB media.
- 7.1.5. Swirl to mix the LB-antibiotic solution
- 7.1.6. Store at 4C

7.2. **Antibiotics**

7.2.1. **Ampicillan (150 mg/ml)**

- 7.2.1.1. Add 7.5 grams of Ampicillan to 50 ml of dH₂O in a 100 ml beaker
- 7.2.1.2. Mix on stir plate until dissolved completely
- 7.2.1.3. Sterile filter using a 0.22 µm filter unit
- 7.2.1.4. Aliquot to 1.5 ml eppendorf tubes and store at -20°C

7.2.2. **Kanamycin (30 mg/ml)**

- 7.2.2.1. Add 1.5 grams of Kanamycin to 50 ml of dH₂O in a 100 ml beaker
- 7.2.2.2. Mix on stir plate until dissolved completely
- 7.2.2.3. Sterile filter using a 0.22 µm filter unit
- 7.2.2.4. Aliquot to 1.5 ml eppendorf tubes and store at -20°C

7.2.3. **Chloramphenicol (34 mg/ml)**

- 7.2.3.1. Chloramphenicol is light sensitive. Aluminum foil must be wrapped around all containers and the solution should be made in room with minimum light.
- 7.2.3.2. Prepare a 50% ethanol solution by mixing 1:1 (v/v) of 100% ethanol and dH₂O

- 7.2.3.3. Add 340 mg of chloramphenicol to 10 ml of 50% ethanol solution in a 25 ml beaker
- 7.2.3.4. Mix on stir plate until dissolved completely
- 7.2.3.5. Sterile filter using a 0.22 μm filter unit
- 7.2.3.6. Aliquot to 1.5 ml eppendorf tubes and store at -20°C

7.3. LB Plates +/- Antibiotic:

- 7.3.1. Add 7.75 grams of premixed LB, 7.50 grams of Bactoagar to 500 ml of dH_2O into a 2L Erlenmeyer flask with a stir bar
- 7.3.2. Autoclave for 30 minutes
- 7.3.3. Cool the LB-agar with continuous stirring till $\sim 50^{\circ}\text{C}$ (this is when you can pick up the flask without a glove)
- 7.3.4. If desired add 500 μl of antibiotic (ampicillin (150 mg/ml), kanamycin (30 mg/ml) or chloramphenicol (34 mg/ml)) to the cooled LB-agar.
- 7.3.5. Stir for another 1 – 2 minutes to ensure antibiotic mixed into the LB-agar.
- 7.3.6. Pour ~ 10 – 15 ml of LB-agar into each Petri dish plate
- 7.3.7. Let the plates cool, with cover partly open for ~ 1 hour
- 7.3.8. Store the plates inverted at 4°C to prevent condensation.

8. REFERENCED DOCUMENTS

- 8.1. Operational Manual, Getinge 533LS Autoclave
- 8.2. Instructions for Sterile Filter Units