

**iGEM TU/e 2018**  
Biomedical Engineering

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## **Preparation of culture media, agar plates, antibiotics and general necessities**

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Preparation of culture media, agar plates,  
antibiotics and general necessities

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# 1 Preparation of Culture Media

## 1.1 Luria-Bertani (LB) Medium

**Estimated bench time:** 15 minutes

**Estimated total time:** 3 hours

**Purpose:** This medium is used for small cultures of competent cells containing desired DNA.

### 1.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- dH<sub>2</sub>O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

### 1.1.2 Setup & Protocol

- For 1 l LB medium, the correct amounts are:
  - 10 g NaCl
  - 10 g peptone
  - 5 g yeast extract
- Collect them in in a bottle and add 1 l of dH<sub>2</sub>O.
- Autoclave the LB medium at 121 °C for 20 minutes (sterilisation).

## 1.2 2YT

**Estimated bench time:** 15 minutes

**Estimated total time:** 3 hours

**Purpose:** This medium is used for the protein expression of competent cells with more than one plasmid.

### 1.2.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- dH<sub>2</sub>O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

### 1.2.2 Setup & Protocol

- For 1 l LB medium, the correct amounts are:
  - 10 g yeast extract
  - 16 g peptone
  - 5 g NaCl
- Collect them in in a bottle and add 1 l of dH<sub>2</sub>O.
- Autoclave the 2YT at 121 °C for 20 minutes (sterilisation).

## 2 Agar Plates

### 2.1 Preparation of LB-agar

**Estimated bench time:** 15 minutes

**Estimated total time:** 3 hours

**Purpose:** Making LB-agar for the pouring of LB-agar plates.

#### 2.1.1 Materials

- Autoclave
- Autoclave tape
- Bacto-agar
- Balance
- Bottle (glassware)
- H<sub>2</sub>O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

#### 2.1.2 Setup & Protocol

With 200 ml LB-agar you can make 8 plates (1 l for ~40 plates).

- For 200 ml LB-agar, the correct amounts are:
  - 1 g yeast extract
  - 2 g NaCl
  - 2 g peptone
  - 3 g bacto-agar
- Collect them in in a bottle and add 200 ml of H<sub>2</sub>O.
- Autoclave the LB-agar at 121 °C for 20 minutes (sterilisation).

### 2.2 Pouring the plates

**Estimated bench time:** 20 minutes

**Estimated total time:** 80 minutes

**Purpose:** Making LB-agar plates where bacteria with an additional plasmid can grow.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

### 2.2.1 Materials

- Antibiotic stock
- Autoclaved LB-agar
- Bunsen burner
- Petri-dishes
- Pipettes and tips

### 2.2.2 Setup & Protocol

- After autoclaving the LB-agar (at 121 °C for 20 minutes), let the agar cool down to ~50 °C (autoclave can be opened at 90 °C). Make sure the agar does not start solidifying.
- Add antibiotic stock (200 µl for 200 ml) to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri-dishes until the bottom is well covered. Work near the Bunsen burner flame.
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench.
- Transfer the plates to a bag, in which they should be placed upside down.
- Store the plates in the fridge (4 °C).

## 3 Antibiotic stocks

### 3.1 Ampicillin stock (50 mg/ml)

**Estimated bench time:** 15 minutes

**Estimated total time:** 15 minutes

**Purpose:** Making antibiotic stock, necessary for LB-agar plates and the LB medium for small culturing.

#### 3.1.1 Materials

- 0.22 µm filter
- Ampicillin
- Balance
- Eppendorf tubes
- Falcon tube
- MiliQ
- Syringe
- Vortex

#### 3.1.2 Setup & Protocol

- Dissolve 0.25 g ampicillin in 5 ml MiliQ.
- Mix/vortex so that all the ampicillin goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 °C under dark conditions.

## 3.2 Chloramphenicol stock (35 mg/ml)

**Estimated bench time:** 15 minutes

**Estimated total time:** 15 minutes

**Purpose:** Making antibiotic stock, necessary for LB-agar plates and LB medium for small culturing.

### 3.2.1 Materials

- 0.22 µm filter
- Balance
- Chloramphenicol
- Eppendorf tubes
- Ethanol (100%)
- Falcon tube
- Syringe
- Vortex

### 3.2.2 Setup & Protocol

- Dissolve 0.136 g of chloramphenicol into 4 ml 100% ethanol.
- Mix/vortex so that all the chloramphenicol goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 °C.

## 4 General necessities

### 4.1 50% Glycerol

**Estimated bench time:** 10 minutes

**Estimated total time:** 3 hours

**Purpose:** Preparing glycerol for a glycerol stock, which can be made for long term storage of bacteria.

#### 4.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- Glycerol (100%)
- MilliQ

### 4.1.2 Setup & Protocol

- Add 10 ml of glycerol (100%) to a bottle.
- Add 10 ml of MilliQ to the glycerol.
- Autoclave the glycerol stock at 121 °C for 20 minutes (sterilisation).

## 4.2 Sterile H<sub>2</sub>O

**Estimated bench time:** 5-10 minutes

**Estimated total time:** 3 hours

**Purpose:** Making sterile H<sub>2</sub>O (nuclease free water), necessary for making dilutions.

Aliquot needs to be done sterile, thus disinfect your hands and work near a Bunsen Burner.

### 4.2.1 Materials

- Autoclave
- Autoclave tape
- Bottle (glassware)
- Bunsen Burner
- Eppendorf tubes (sterile)
- MilliQ
- Pipette and tips

### 4.2.2 Setup & Protocol

- Fill a bottle with 100 ml MilliQ.
- Autoclave the MilliQ at 121 °C for 20 minutes (sterilisation).
- Optional: aliquot a part into sterile Eppendorf tubes for easy usage.

## 4.3 Sterile Eppendorf, PCR and sequencing tubes

**Estimated bench time:** 10 minutes

**Estimated total time:** 3 hours

**Purpose:** Making the Eppendorf tubes, PCR tubes and sequencing tubes sterile so that they can be used during experiments.

### 4.3.1 Materials

- Autoclave
- Autoclave tape
- Beaker
- Eppendorf tubes
- PCR tubes
- Sequencing tubes
- Tin foil

### 4.3.2 Setup & Protocol

- Fill a beaker with the tubes, for each type, use another beaker.
- Cover the upside with tin foil.
- Autoclave the beakers with the tubes at 121 °C for 20 minutes (sterilisation). Use a dry sterilization program or let the tubes dry in an incubator set at 50 °C.