iGEM2013 – Microbiology – BMB – SDU	
Title: Preparation of agar plates with chloramphenicol **SOP number:** SOP0018_v01 **Version number:** 01	**Date issued:** 2013.06.28 **Review date:** **Written by: MHK**

1. Purpose

To prepare agar plates with chloramphenicol

2. Area of application

All *E. Coli* cells with chloarmphenicol resistance.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Autoclave	Laboraory class 1, chemical room	●	
Incubator	Laboratory class 1, PCR/gel room	●	
Pipette aid xp	Laboratory class 1	●	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Green pipette tips		Contact lab-manager	Micro storage	
LA media		SOP0016		
Petri dishes				

Ethanol				
500mg Chloramphenicol				Use fume rood
25mL pipette tube		Conctact lab manager	Micro storage	
5mL syringe		Conctact lab manager	Micro storage	
Needle for syringe		Conctact lab manager	Micro storage	
100 mL Erlenmeyer flask				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0016 Making LB and LA media

7. Environmental conditions required

8. Procedure

1. Prepare 500 mL LB agar according to SOP0016 “Making LB and LA media”. (According to iGEM: Adjust pH to 7.5 if necessary with 1M NaOH).
2. Autoclave media (with caps loosened!). Do not open autoclave to remove media before it has cooled down to 80 deg C.
3. Place media in incubator at 60 deg C.
4. Prepare chloramphenicol unless liquid stocks with a concentration of 17,5 mg/mL chloramphenicol in ethanol are available. Else go to 8.5
    1. Add 25 mL of ethanol to the Erlenmeyer flask using the Pipette aid and pipette tube.
    2. Add 4 mL of ethanol to the 500 mg chloramphenicol powder using the syringe and needle. Swirl until completely dissolved.
    3. Transfer the chloramphenicol solution to the Erlenmeyer flask, again using the syringe and needle. Swirl to mix.

5. When media has cooled down to 60 deg C add 1mL chloramphenicol solution to the 500 mL LA (concentration of liquid stocks chloramphenicol: 17,5 mg/ml).
6. Swirl to mix; try not to make many bubbles.
7. Fill petri dishes with agar and cap. Once set, store in refrigerator for future use.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks

10. Time consumption

- Total-time 3 hours.
- Hands-on-time 45 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.28 / MHK	01	The SOP has been written

12. Appendices

Recommended concentrations of chloramphenicol according to iGEM

http://parts.igem.org/Help:How_to_make_agar