iGEM 2017 – Microbiology – BMB – SDU

Title: LA plates with antibiotic	**Date issued:** 2012.11.26
SOP number: SOP01	**Review date:** 2017.09.10
Version number: 03	**Original by:** TKR
	Modified by: FN, EG

1. Purpose

To make LA plates with selection

2. Area of application

This procedure is valid for all antibiotics

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Refrigerator	Laboratory (class 1)	●	
Small autoclave	Laboratory (class 1) – V18-405-0	●	121°C
Pipette (p1000, 100)		●	
Measuring pitcher 1 L	Glass hallway	●	

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
LA	1% Tryptone 1% NaCl 0.5% Yeast extract 1.5% agar	Oxoid Sigma-Aldrich Merck Difco agar from BD	Anne-Mette Or V18-405-0	
Appropriate antibiotic	Manufacture/cat#, stock solution conc.; Stock solution date of creation, dilution in medium		V18-405-0	
Plastic Petri dishes		Contact lab-manager (Sarstedt)	BMB storage	
Green Pipette tips		Contact lab-manager	Micro storage	
Blue Pipette tips		Contact lab-manager	Micro storage	
Plastic bag	LDPE standardpose	CC&CO Living Office Lev varenr: 281615		

5. QC – Quality Control

Calculation of antibiotic concentration

$$Antiobiotic\ stock\ concentration \times Required\ volumen\ of\ antibiotica$$

$$= Desired\ concentration\ of\ antibiotica \times Total\ volumen\ of\ media$$

$$\Rightarrow$$

$$Required\ volume\ of\ antibiotica = \frac{Desired\ concentration\ of\ antibiotica \times Total\ volumen\ of\ media}{Antiobiotic\ stock\ concentration}$$

The temperature of the LA media may not be more than 60°C when the antibiotic is added

It is possible to leave the autoclaved LA media at 58°C up till 24 hours before adding antibiotic and pouring into plates.

Note that some antibiotic are decomposed over time, specially ampicillin

6. List of other SOPs relevant to this SOP

iGEM2017_SOP14_v02_EG_Table_Autoclave

7. Environmental conditions required

8. Procedure

Mix components of LA in glass bottle and autoclave (amount is labeled on)

8.1 Add the appropriated amount of antibiotic when the temperature is between 50-60 °C, see appendix
8.2 Fill up the required amount of petri dishes (between 15-25 ml LA in each)
8.3 Dry on table for at least 2 hours, can stand ON
8.4 Note type and concentration of antibiotic and the date at the bottom of the dishes
8.5 Place in bag in the refrigerator until use

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
LA media rest		GMO waste	

10. Time consumption

- Total-time 3½ hours if the dishes are dried 3 hours
- Hands-on-time ½ an hour

11. **Scheme of development**

Date / Initials	Version No.	Description of changes
12.11.26 / TKR	01	The SOP has been written
13.05.22 / MM & TK	01	The SOP has been approved
17.04.28 / FN	02	The SOP has been modified
17.05.01 / EG	02	The SOP has been approved
17.09.10 / EG	03	The SOP has been modified

12. **Appendices**

- **Addition of antibiotic**
    - Chloramphenicol
        - Add 1 µL of 30 mg/mL chloramphenicol per mL LA/LB to achieve 30 µg/mL.
    - Ampicillin
        - Add 1.0 µL of 100 mg/mL ampicillin per mL LA/LB to achieve 100 µg/mL.
    - Kanamycin
        - Add 1.0 µL of 50 mg/mL kanamycin per mL LA/LB to achieve 50 µg/mL.