

iGEM 2013 – SDU

Title: LA plates with antibiotic

Date issued: 2012.11.26

SOP number: SOP0007_v01

Review date: 2013.12.01

Version number: 01

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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

$$\begin{aligned} & \textit{Antibiotic stock concentration} \times \textit{Required volumen of antibiotica} \\ & = \textit{Desired concentration of antibiotica} \times \textit{Total volumen of media} \end{aligned}$$

=>

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

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

JMJ_SOP00012_v01_TK_Small autoclave

7. Environmental conditions required

8. Procedure

- 8.1 Mix components of LA in glass bottle and autoclave
- 8.2 If using premade LA from Anne-Mette bottles under ½ L can be warmed in a microwave the rest should be autoclaved.
- 8.3 If microwave is used, place the bottle in a measuring pitcher with water
- 8.4 Unscrew the lid
- 8.5 Set the microwave to max power 10 min until the media is melted
- 8.6 Keep an eye on the bottle the last 5 min
- 8.7 Add the appropriated amount of antibiotic when the temperature is between 50-60 °C
- 8.8 Fill up the required amount of petri dishes (between 15-25 ml LA in each)
- 8.9 Dry on table for at least 3 hours, can stand ON
- 8.10 Note type and concentration of antibiotic and the date at the bottom of the dishes
- 8.11 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 / TK	01	The SOP has been written
13.05.22 / MM & TK	01	The SOP has been approved

12. Appendixes