iGEM2017 – Microbiology – BMB – SDU

Title: Making LB and LA media **SOP number:** SOP12 **Version number:** 03	**Date issued:** 2013.06.26 **Review date:** 2017.09.10 **Original by:** PRA **Modified by:** EG

1. Purpose

To make LB or LA media

2. Area of application

All organisms that live in/on LB/LA media

3. Apparatus and equipment

Apparatus/ equipment	Location (Room number)	Check points	Criteria for approval/rejection
Weight	Laboratory (class 1) – Chemical room	●	
Autoclave	Laboratory (class 1) – Chemical room	●	

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

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Tryptone		Contact lab manager	Micro chemical room	
Sodium chloride (NaCl)		Contact lab manager	Micro chemical room	
Yeast extract		Contact lab manager	Micro chemical room	
500mL/1000 mL bottle with white cap			V11-504a-1 V10-504a-1	
Autoclave tape		Contact lab manager	Micro chemical room	
Agar		Contact lab manager	Micro chemical room	

5. QC – Quality Control

Check using a negative and positive control if the compounds added to the LA media function by streaking bacteria onto one of the plates and leave ON at appropriate temperature.

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

8.1 Take an appropriate (e.g. 500 mL) blue cap bottle and fill approximately ⅓ with distilled water.
8.2 Add the appropriate amount LB or LA as described on the container
8.3 Close the bottle tightly and mix thoroughly by shaking
8.4 Add distilled water to fill the bottle
8.5 Screw the cap on the bottle, and loosen it a bit (¼-½ turn)
8.6 Put autoclave tape on the cap and a bit down the side of the bottle (1 piece only)

8.7 Autoclave the media following SOP14
8.8 Optional: add antibiotic or other compounds in wanted concentration (be aware that many compounds are temperature sensitive. Therefore, do not add too early)
8.9 When cold enough to hold without getting burned, distribute into plates by following SOP01.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Agar plate	N/A	Bio waste (yellow bags)	
LB media	N/A	Bio waste (liquid media container in growth room)	

10. Time consumption

- Total-time 3 hours.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.26 / PRA	01	The SOP has been written
17.05.05 / EG	02	The SOP has been modified
17.05.05 / SJ	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 µL of 100 mg/mL ampicillin per mL LA/LB to achieve 100 µg/mL.
  - Kanamycin
    - Add 1 µL of 50 mg/mL kanamycin per mL LA/LB to achieve 50 µg/mL.