iGEM2013 – Microbiology – BMB – SDU

Title: Making LB and LA media	**Date issued:** 2013.06.26
SOP number: SOP0016_v01	**Review date:** 2013.06.26
Version number: 01	**Written by:** Patrick Rosendahl Andreassen

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/1000mL 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 For 1L LB media add following to a 1000mL white cap bottle:
- 8.1.1 10 g tryptone
- 8.1.2 5 g yeast extract
- 8.1.3 10 g NaCl
- 8.1.4 Add distilled water to 1L

8.2 For 1L (ca. 40 plates) LA medium add following to a 1000mL white cap bottle:
- 8.2.1 10 g tryptone
- 8.2.2 5 g yeast extract
- 8.2.3 10 g NaCl
- 8.2.4 1.5 % agar (1% = 1g/100mL)
- 8.2.5 Add distilled water to 1L

8.3 Screw the cap on the bottle, and loosen it a bit

8.4 Put autoclave tape on the cap and a bit down the side of the bottle (1 piece only)

8.5 Autoclave the media

8.6 After autoclavation let the LA media cool down before distributing it into plates.

8.7 Optional: add antibiotic or other compounds in wanted concentration (be aware that many compounds are temperature sensitive. Do not add too early)

8.8 When cold enough to hold without getting burned, distribute into plates. Make sure the surface is smooth (no bubbles)

8.9 Leave them to cool and dry ON at RT (be aware that some compounds are light sensitive)

8.10 Put into a bag to prevent the plates from drying out and store at 5^{o}C.

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

12. Appendixes