

<h2>iGEM 2016 – SDU</h2>	
Title: Nile Red agar plates	Date issued: 2016.07.04
SOP number: SOP0036	Review date: 2016.10.11
Version number: 01	Written by: Joel Vej-Nielsen & Jakob Rønning

1. Purpose

Creating differential media that will reveal PHB producing bacterial cultures.

2. Area of application

This SOP is valid only for Nile red

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Refrigerator	Laboratory (class 1)		
Autoclave	Incubation room		121°C
Pipette (p1000)	Hallway storage (1. Floor)		
Measuring pitcher	Hallway storage (-1. Floor)		Appropriate size
Scale	Chemical room		

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	Chemical room	
Appropriate antibiotic		Contact Lab-manager	V18-405-0	
Petri dishes		Contact Lab-manager	BMB storage	
Blue Pipette tips		Contact Lab-manager	Micro Storage	
Plastic bag	LDPE standard bag	CC&CO Living Office		
DMSO			Chemical room	Use in fume closet
Nile red		Sigma-Aldrich		Avoid light

5. QC – Quality Control

The temperature of the LA media may not be more than 60°C when the antibiotic and Nile red 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

7. Environmental conditions required

8. Procedure

- 8.1 Mix components of LA in glass bottle and autoclave
- 8.2 Mix 1 ml and 0,25 mg nile red. (keep from light)
- 8.3 Add appropriate antibiotic and nile red solution when temperature is between 50-60°C
- 8.4 Fill up the required amount of petri dishes
- 8.5 Dry on table for 3 hours, or leave over night
- 8.6 Note that it is a nile red plate, the antibiotic used and the date on the bottom of the plates.
- 8.7 Place in bag, wrap in alu foil and store at 4°C until use.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One use plastic		GMO yellow waste	

10. Time consumption

- Total-time 3h 15 min.
- Hands-on-time 20 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.07.04 / JVN & JR	01	The SOP has been written
16.10.11 / JVN & JR		The SOP has been reviewed

12. Appendixes