iGEM2013 – Microbiology – BMB – SDU	
Title: ON culture of *E. coli* **SOP number:** SOP0001_v01 **Version number:** 01	**Date issued:** 2012.10.25 **Review date:** 2013.12.01 **Written by:** Michelle Madelung

1. Purpose

To prepare ON culture of E.coli for use in experiments

2. Area of application

This procedure is valid for all E. coli ON cultures

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Cr
Incubator	Laboratory (class 1) - V16-407-2 Laboratory (class 2) – V15-501b-2	• Preheated	37
Spectrometer	Laboratory (class 1) - V16-407-2 Laboratory (class 2) – V15-501a-2	• Set to wavelength 600	
Vortex	Laboratory (class 1) Laboratory (class 2) – V15-501a-2	•	
Pipette boy		• Remember to recharge	
Racks		•	
Sterile glass culture tube	Laboratory (class 1) – opposite elevator Laboratory (class 2) – V16-501a-2 filing cabinet	•	

Refrigerator		●	
Pipettes (p1000,200)		●	

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

Name	Components	Supplier / Cat. #	Room (hallway storage)	Sa
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-405-0	
Appropriate antibiotic if needed				
5 ml graduated pipettes		Fisher Scientific / CCI 4487	Micro storage	
Cuvettes		Contact lab-manager	BMB storage	
Blue pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Plate Bag		Contact lab-manager	BMB storage	

5. QC – Quality Control

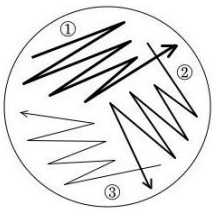
When measuring OD_{600}, measurements can't go above 0.300, if this is the case dilute solution with medium 10 times.

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

1. Take an agar plate with appropriate medium and antibiotic
2. Scrap surface of frozen bacterial stock



3. Streak this bacterial stock onto agar plate (primary streak)
4. Take a new pipette tip streak again (secondary steak)
5. Take a new pipette tip streak again (tertiary streak)
6. Place the plate in a plate bag
7. Leave 16 hours in incubator set to 37 ˚C
8. Move plate to refrigerator (4 ˚C)
9. Fill 5 ml medium in culture tube
10. Add antibiotic to appropriate concentration
11. Take single colony from agar plate
12. Vortex medium with colony
13. Place culture tube in incubator set to 37 ˚C with aeration (155 rpm)
14. Leave 16 hours
15. Add 1 ml medium to a cuvette and calibrate spectrometer
16. Add 0.9 ml medium to a cuvette and 0.1 ml ON culture and mix
17. Measure OD

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
ON Culture		Liquid bacterial waste	
Once use plastic		GMO yellow waste	

10. Time consumption

- Total-time 30 hours and 20 min.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
12.10.25 / MM	01	The SOP has been written
13.01.02 / MM & TK	01	The SOP has been approved

12. **Appensdixes**