

<h2>iGEM 2016 – SDU</h2>	
Title: Ethylacetat PHB recovery	Date issued: 2016.08.18
SOP number: SOP0032	Review date: 2016.10.11
Version number: 01	Written by: Joel Vej-Nielsen & Jakob Rønning

1. Purpose

Extracting PHB from bacteria with ethyl acetate.

2. Area of application

This procedure is valid for all *E. coli* strains that produce PHB.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Centrifuge	Incubation room (class 1) – V18-404b-0		Can centrifuge 250 ml each type x6
Waterbath	Incubation room		With variable temperature and mixing
50 ml tube rack	Hallway storage (1. Floor)		
Vortex			
Scale	Incubation room (class 1) – V18-404b-0		

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Ethyl acetate		Sigma		Handle in fume closet
Distilled water			Micro storage	
50 mL falcon tubes		Contact Lab-manager	Micro storage	
Acetone				Handle in fume closet
100 – 200 mL flask			Micro storage	
250 or 500 mL centrifuge tubes		Incubation room (class 1) – V18-404b-0		

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0001 – ON culture

SOP0031 – TB media

7. Environmental conditions required

8. Procedure

- 8.1 Inoculate arbitrary volume of LB with PHB producing plastic and incubate for 48 h at 37,5°C with 700 rpm.
- 8.2 Centrifuge cells at 10,000 rpm for 5 min.
- 8.3 Discard supernatant and resuspend cells in 10ml ethyl acetate for each gram biomass.
- 8.4 place cells in water bath at room temperature and set the temperature of the bath to 35 °C and turn on mixing/stirring.
- 8.5 when the water bath has reached 35 °C, incubate cells for 24 hours at room temperature.
- 8.6 Centrifuge cells and collect the supernatant
- 8.7 Precipitate with acetone.
- 8.8 The precipitated biopolymer was washed with distilled water.
- 8.9 Centrifuge and remove as much supernatant as possible
- 8.10 Leave PHB at room temperature till dried.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
Supernatant		Liquid bacterial waste	
Once use plastic		GMO yellow waste	
Acetone		C waste	
Ethyl acetate		C waste	

10. Time consumption

- Total-time 74h 30 min.
- Hands-on-time 2 h 30 min.

11. Scheme of development

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

12. Appendixes