

## iGEM 2016 – SDU

**Title:** Chloroform PHB recovery

**Date issued:** 2016.09.19

**SOP number:** SOP0034

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**Version number:** 01

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### 1. Purpose

Extracting PHB from bacteria with acetone and chloroform.

### 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 50 ml falcon tubes
Freezer	Basement		Has to be at -80°C
Flask	Mikro Glasware		Has to be sterile
Waterbath	Incubation room		With variable temperature and mixing
50 ml tube rack	Hallway storage (1. Floor)		
Vortex			

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

Name	Components (Concentrations )	Manufacturer / Cat. #	Room	Safety considerations
Acetone		AppliChem	Anne Mette, RT	Handle in fume closet
chloroform		Sigma	Micro storage	Handle in fume closet
Freezing tubes		Contact Lab-manager	Micro storage	

## 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 500-700 rpm.
- 8.2 Centrifuge cells at 10.000 rpm for 15 min.
- 8.3 Gather the bio mass in 50 ml falcon tubes, and centrifuge the gathered biomass at 10,000 min for 5 min.
- 8.4 Discard supernatant and lyophilize cells with liquid nitrogen and store at -80°C.
- 8.5 Resuspend cells in 10ml acetone for each gram wet biomass in a sterile flask.
- 8.6 Incubate cells in waterbath at 25°C for 24 hours and turn on mixing.
- 8.7 Recover biomass by filtration.
- 8.8 Dry biomass in a fume closet.
- 8.9 Grind the dried biomass with mortar and pestle.
- 8.10 Resuspend in 10ml chloroform (at 61°C) (or 1,2 – chloroethane (at 83°C)) for each gram of biomass and incubate for 1h in waterbath.
- 8.11 Residual biomass is removed by filtration.
- 8.12 The PHB solution is rotary evaporated until it reaches a volume around 40% of the added chloroform volume.
- 8.13 The PHB solution is mixed with 10 ml cold ethanol and stirred while kept cold for 10 min.
- 8.14 Lastly, PHB is recovered by filtration, pressed to remove ethanol and incubated at 60°C for 24 h under fume.

## 9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
Supernatant		Liquid bacterial waste	

Once use plastic	GMO yellow waste
<b>Chloroform</b>	B
<b>Acetone</b>	C

## 10. Time consumption

- Total-time 80h.
- Hands-on-time 8 h.

## 11. Scheme of development

Date / Initials	Version No.	Description of changes
16.08.19 / JVN & JR	01	The SOP has been written
16.10.10 / JVN & JR	01	The SOP has been approved

## 12. Appendixes