

iGEM 2016 – SDU	
Title: PHB extraction with sodium hypochlorite	Date issued: 2016.09.19
SOP number: SOP0044_v01	Review date: 2016.10.18
Version number: 01	Written by: Joel Vej-Nielsen & Jakob Rønning

1. Purpose

To extract PHB from bacteria with hypochlorite.

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	Incubationsroom		50mL 4000rpm
1000 mL pipette			
Vortex			
Centrifuge			Can centrifuge 250 mL or 500 mL x6 tubes at same time.
Freezer			Has to be at 80°C
50 mL tube rack			
Single use plastic			
Heatbath	under		For letting the PHB dry overnight
fume scale			

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Sodium hypochlorite	6-14%	sigma	Micro storage	
Distilled H ₂ O				
Ethanol	70 %			

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0001 – Culture ON

SOP0031 – TB media

7. Environmental conditions required

8. Procedure

- 8.1 Inoculate arbitrary volume of TB with PHB producing plastic and incubate for 48 h at 37,5 with 500-700 rpm.
- 8.2 Centrifuge cells at 10.000 rpm for 5 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.
- 8.5 The freeze dried cells were re-suspended in a 7 % sodium hypochlorite solution.
- 8.6 The first 20 minutes after the re-suspension in hypochlorite, which is carried out at room temperature, with the lids only slightly attached.
- 8.7 The lids are tightened and the tube is shaken at 37°C for an hour, the air pressure is released every 5-10 minutes.
- 8.8 Centrifuge at 4000xg at 4°C for 5 minutes, discard the supernatant.
- 8.9 Re-suspend the pellet in 70% ethanol and centrifuge at 4000xg at 4°C for 5 minutes and discard the supernatant.
- 8.10 Re-suspend the pellet in distilled water and centrifuge at Centrifuge at 4000xg at 4°C for 5 minutes and discard the supernatant carefully.
- 8.11 Allow to dry overnight at 40-60°C.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
Supernatant		Liquid bacterial waste	
Once use plastic		GMO yellow waste	
Sodium hypochlorite		X	

10. Time consumption

- Total-time 48 h.
- Hands-on-time 2 h 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.09.19 / JVN & JR	01	The SOP has been written
16.10.13/PA		The SOP has been approved

12. Appendixes