

<h2>iGEM 2016 – SDU</h2>	
Title: PHB extraction with triton x-100 and hypochlorite	Date issued: 2016.09.19
SOP number: SOP0033	Review date: 2016.10.11
Version number: 01	Written by: Joel Vej-Nielsen & Jakob Rønning

1. Purpose

To extract PHB from bacteria with triton x-100 and 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	Incubation room		50mL 4000rpm
Centrifuge			Can centrifuge 250 mL or 500 mL x6 tubes at same time.
1000 mL pipette			
Vortex			
Waterbath			
heatbath	under		For letting the PHB dry overnight
fume			
Scale			
50 mL falcon tubes			
50 mL tube rack			
Single use plastic			

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Triton X-100	1% v/v in PBS		Micro storage	
PBS				
Sodium hypochlorite	6-14%	sigma	Micro storage	Handle under fume
Distilled H ₂ O				
Ethanol	70 %			

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 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 Every step beneath should be centrifuged at 4000 rpm for 5-10 min and afterward the supernatant should be poured off.
- 8.6 Resuspend 5 ml PBS for each 1 g wet biomass.
- 8.7 Centrifuge and resuspend the pellet in 5ml triton X-100 I 30 min.
- 8.8 Centrifuge and resuspend in 5mL PBS for each 1 g wet biomass.
- 8.9 Centrifuge, add 40 ml sodium hypochlorite (7%) for each wet biomass and incubate at 30°C for an 1 hour
- 8.10 Centrifuge, wash with 5ml distilled water and 5 ml 70% EtOh twice.
- 8.11 Leave PHB to dry at 40-60 °C overnight.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
Supernatant		Liquid bacterial waste	
Once use plastic		GMO yellow waste	
Hypochlorite		X	
Triton x-100		C	

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 / JVN & JR		The SOP has been reviewed

12. Appendixes