

<b>iGEM 2016 – SDU</b>	
<b>Title:</b> Gas chromatography	<b>Date issued:</b> 2016.10.04
<b>SOP number:</b> SOP0045	<b>Review date:</b> 2016.10.11
<b>Version number:</b> 01	<b>Written by:</b> Joel Vej-Nielsen

## **1. Purpose**

Determination of intracellular PHB concentration.

## **2. Area of application**

This procedure is valid for all strains of PHB producing cells.

### 3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Heater	FKF		Heat to 100 °C
Gas chromatograph	FKF		
Centrifuge			
Glas vials			Able to hold in steam.

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Benzoic acid		Sigma Aldrich	Chemical room	
PHB	pure	Sigma Aldrich		
Methanol		Sigma Aldrich	FKF	Use in fume closet
$H_2SO_4$	100%		Chemical room	Use in fume closet
Chloroform		Sigma Aldrich	Chemical room	Use in fume closet
Distilled water				

## 5. QC – Quality Control

## 6. List of other SOPs relevant to this SOP

SOP0001 – ON culture

## 7. Environmental conditions required

## 8. Procedure

- 8.1 Spin down ON culture of cells and lyophilize 20 mg of biomass.
- 8.2 Add sulfuric acid to methanol to create a 7% solution.
- 8.3 Add variable amount of benzoic acid as internal standard
- 8.4 Dissolve appropriate amount of plastic in 2 mL of sulfuric acid solution.
- 8.5 Dissolve 20 mg of prepared cell samples in 2 mL of sulfuric acid solution each.
- 8.6 Add 4 mL chloroform to each solution
- 8.7 Boil samples at 100°C for 2,5 hours
- 8.8 Let samples cool to room temperature
- 8.9 Add 4 mL distilled water and cool quickly.
- 8.10 Make measurements on gas chromatograph

## 9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
Chloroform		B	

## 10. Time consumption

- Total-time 2h
- Hands-on-time 1h

## 11. Scheme of development

<b>Date / Initials</b>	<b>Version No.</b>	<b>Description of changes</b>
16.10.04 / JVN	01	The SOP has been written
16.10.11 / JR		The SOP has been revised

## 12. Appendixes