Safety Documentation

Please select the forms you require by selecting the check boxes below.
You can select more than one.

☑ **Risk Assessment** ☑ **Method Statement** ☑ **Chemicals COSHH**

Once you have made your selections, scroll down and complete the forms.

Buttons: [+] will add a row to a list [X] will delete a row from a list

You may save this file to a local drive at any time.
When you have finished, save the file to a local drive and email it to your supervisor for authorisation.

Supervisors - There is a sign-off section at the end of the document set that must be completed.

Staff may "self authorise", (as a supervisor), but the forms must still be submitted to the DSO for approval.

IMPORTANT:

YOU ***MUST NOT*** START ANY PRACTICAL WORK UNTIL THESE FORMS HAVE BEEN RETURNED TO YOU WITH **BOTH** YOUR SUPERVISOR's AND DSO's APPROVAL SIGNATURES ATTACHED.

Please complete these fields

School or Service	Wolfson School of Mechanical, Electrical and Manufacturing Engineering
Department	Centre for Biological Engineering
Originator name	Sotiria Toumpaniari
email address	s.toumpaniari@lboro.ac.uk
Location	H25, H34
Project / Activity / Task	Delipidisation and deglycosation of porcine tissues
Supervisor Name	Sotiris Korossis

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Loughborough University
Centre for Biological Engineering

Risk Assessment

Reference: SAF/MEME 6512

Location: H25, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Delipidisation and deglycosation of porcine tissues

Is this process risk assessment for a : ☑ Laboratory / Workshop ○ General use

Category 1: Machinery & work equipment:				
Design and Construction	Mechanical hazards	Electrical hazards	Radiation hazards	+
N/A	N/A	Electrical test lables current	Heat(Inc. IR)	x
Category 2: Workplace				+
Slips/Trips/Falls on the level				x
Category 3: Hazardous and/or Harmful substances				+
Irritant substances				x
Category 4: Work activity				+
Lone working out of hours				x
Category 5: Work organisation				+
N/A				x

Explain the risks associated with these hazards

People / Groups at risk: Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score
Slips/Trips/Falls on the level	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Organise room to have nothing on the floor that can be a trip hazard. Reduce movement between labs if possible.	Significantly	Significantly	x
		Residual Risk	Low
People / Groups at risk: Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score
Aerosols/splashes from irritant substances	Very Harmful	Likely	Unacceptable
What are the control measures?	Lowers Impact	Lowers Probability	+
Work in fume hood	Significantly	Significantly	x
Wear nitrile gloves	Significantly	Significantly	x

Process Risk Assessment Form (Continued)

	Residual Risk
	Low

+ Add another Risk

Who may be at risk as a result of this activity?

Personnel Group	Maximum (Task setup/ Re-configuration)	High (Performing the task)	Medium (Observing the task)	Low (Present, but not involved)	Lone Working (Out of hours)	No Exposure Permitted	Total
Academic Staff	1	0	0	0	0	0	1
Technical Staff	0	0	0	0	0	0	0
Research Staff (PDRA)	0	1	0	0	0	0	1
Research Students (PhD)	0	2	2	0	0	0	4
Students (Undergraduate / MSc)	0	5	5	0	0	0	10
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0
Total	1	8	7	0	0	0	16

With these controls in place, the risk is:

The activity is LOW RISK - and is effectively controlled

Loughborough University
Centre for Biological Engineering

Safety Method Statement

Reference: SAF/MEME 6512

Location: H25, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Delipidisation and deglycosation of porcine tissues

What equipment will be used in this activity?	+
Orbital shaker	x
Minisart Syringe Filter	x
Syringe	x
Eppendorf tubes	x
Thermomixer	x

What training must be completed to do this activity?	+
CBE code of practice, SOP003, SOP004, SOP037, SOP038, SOP048	x

What chemicals are being used? (These must be included in the COSHH Form)	+
α-Amylase, from porcine pancreas	x
DPBS	x
Lipase type II from porcine pancreas (unpurified)	x
KCl	x
PNGase F	x
Lipase type VI-S from porcine pancreas (purified)	x

Spill and accident procedures.	+
Handling, storage and disposal of chemical waste	x

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
Leave a note with details of the user and name of the chemical asking not to move anything from the area.	x

References.	+
CBE code of practice, SOP003, SOP004, SOP037, SOP038, SOP048	x

Detailed sequential description of the process

Process step	Precautionary measures and comments	+
Prepare Duran bottles where the solutions are going to be made and kept.	Be cautious not to drop glassware and break.	x
Measure the powder using using scales.	Always measure powders under fume hood in H25 or H34 to avoid breathing dust.	x
Pour powders in bottles under fume hood	Always work with powders under fume hood in H25 or H34 to avoid breathing dust.	x
Add liquids in the bottles to make solutions	Handle liquids carefully and have absorbent tissue nearby.	x

Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
When required to modify the pH, add as required sodium hydroxide or hydrochloric acid dropwise and check pH.	Be careful not to pour liquid on the pH meter.	x
Filter sterilise solutions.	Make sure that the receiving container can fit all teh liquid.	x
Add appropriate solutions in samples whilst working in a biosafety cabinet.	Be careful not spilling solution and treat waste according to COSSH forms.	x
Treatment with different solutions can take place on the bench.	Ensure lids are properly closed to ensure sterility of samples.	x

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COSHH Form

Reference: MEME 697

Location: H25, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Delipidisation and deglycosation of porcine tissues

CHEMICAL NAME

α-Amylase, from porcine pancreas

CAS No. 9000-90-2

W.E.L. (Itel / stel)

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential	Hazard Rating
0.25	ml	1	Semi Closed	Lyophilised Solid	☐ Eyes ☑ Skin ☐ Inhaled ☐ Ingested	Low	High

OVERALL RISK: **Low**

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description
H317 May cause an allergic skin reaction.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
	P284 Wear respiratory protection.
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com
	P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/p
	P501 Dispose of contents/ container to an approved waste disposal plant.

Justify the use of this chemical:

How will the precautions listed above be implemented?

Use personal protective equipment- nitrile gloves and lab coat. Use chemical in BSC in H25 or fume hood in CTMF. Avoid breathing vapours, mist or gas. Ensure adequate ventilation.

Special Storage and Containment Measures	Disposal Method
Keep container tightly closed in a dry and well-ventilated place. Store in cool place. Recommended storage temperature 2 - 8 °C.	Yellow stream

How will spillages be dealt with?

Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.
Click here to see spill procedures

Absorbent cloth / tissue

+ Add another chemical

Statement of work (Process to be undertaken)

Show Image

1. Unpurified Lipase treatment with filtration:

Cut a small piece of decellularised pericardium, (1cm^2), and treat it as described below:

- Prepare 500-700 U/ml of lipase solution in Tris buffer 200mM, at pH 7.7. Filter-sterilise the solution prior to use.
- Apply the lipase solution to the tissue, for a total volume of 1000ul, for 24hrs at 37° C with agitation in a 2ml epi tube – use the thermomixer.
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation. Use a new 2ml epi tube per each washing step – use the thermomixer;
- After treatment, store tissue in 1ml of fresh PBS, in a new 2ml epi.

2. Amylase treatment with filtration:

Cut a small piece of decellularised pericardium, (1cm^2), and treat it as described below:

- Prepare 50-100U/ml of amylase solution in 25mM Tris-HCl buffer and 100mM KCl, at pH 7.5. Filter-sterilise the solution prior to use
- Apply the amylase solution to the tissue, for a total volume of 1000ul per sample, for 24hrs at RT with agitation (650rpm), in a 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer;
- After treatment, store tissue in 1ml of fresh PBS, in a new 2ml epi.

3. Amylase + PNGase F treatment with filtration:
Cut a small piece of decellularised pericardium, (1cm^2), and treat it as described below:
- Prepare 50-100U/ml of amylase solution in 25mM Tris-HCl buffer and 100mM KCl, at pH 7.5. Filter-sterilize the solution prior to use
- Apply the amylase solution to the tissue, for a total volume of 1000ul per sample, for 24hrs at RT with agitation (650rpm), in a 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- Prepare PNGase F enzyme according to manufacturer instructions. Filter-sterilize the solution prior to use
- Apply 25-50U/ul PNGase F to the tissue, for a total volume of 500ul, for 24hrs at 37° C with agitation (650rpm), in a new 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- After treatment, store tissue in 1ml of fresh PBS, in a new 2ml epi.

4. Purified Lipase + Amylase + PNGase F with filtration:
Cut a small piece of decellularised pericardium, (1cm^2), and treat it as described below:
- Prepare 500-1000U/ml of lipase solution in Tris buffer 200mM, for 3hrs at pH 7.7 and 37° C. Filter-sterilize the enzymatic solutions prior to use
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- Prepare 60U/ml of amylase solution in 25mM Tris-HCl buffer and 100mM KCl, at pH 7.5. Filter-sterilize the solution prior to use
- Apply the amylase solution to the tissue, for a total volume of 1000ul per sample, for 24hrs at RT with agitation (650rpm), in a 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- Prepare PNGase F enzyme according to manufacturer instructions. Filter-sterilize the solution prior to use
- Apply 25-50U/ul of PNGase F to the tissue, for a total volume of 500ul, for 24hrs at 37° C with agitation (650rpm), in a new 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- After treatment, store tissue in 1ml of fresh PBS, in a new 2ml epi.

5. Purified lipase + PNGase F without filtration of the enzymes:
Cut a small piece of decellularised pericardium, (1cm^2), and treat it as described below:
- Prepare 500-1000U/ml of lipase solution in Tris buffer 200mM, for 3hrs at pH 7.7 and 37° C
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- Prepare PNGase F enzyme according to manufacturer instructions
- Apply 25U/ul of PNGase F to the tissue, for a total volume of 500ul, for 24hrs at 37° C with agitation (650rpm), in a new 2ml epi tube – use the thermomixer
- After treatment, do 3 washes with 1ml of PBS for 10min each at RT, with agitation (650rpm). Use a new 2ml epi tube per each washing step – use the thermomixer
- After treatment, store tissue in 1ml of fresh PBS, in a new 2ml epi.

Personal protection requirements not covered in the precaution statements above.

Closed shoes, and over shoes- only for work in CBE

COSHH Form (Continued)

Sources of information and references	Reference to **existing approved** Risk Assessment
https://www.sigmaaldrich.com/catalog/product/sigma/a3176?lang=en&region=GB	

With the current controls, the risk of using these chemicals is: Low

Supervisor to check that the process involving the safe use of these chemicals has been satisfactorily evaluated

Supervisor and Departmental Safety Office (DSO) Sign-off.

Supervisors

Please check the documents above and if you want to approve them:

1) Electronically sign this document
2) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the DSO.

DSO

Please review the documents above and if you want to approve them:

1) Enter the reference numbers as appropriate
2) Electronically sign this document
3) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the originator

IF YOU DO NOT WANT TO AUTHORISE THE FORMS,

Please do not sign the form, but click the "Not Approved" check-box and return it to the originator by email stating why and what you expect them to do to put it right in the comments box below.

Not Approved ☐

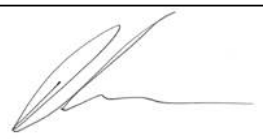
Supervisors Signature: Professor Sotiris Korossis

Digitally signed by Professor Sotiris Korossis
Date: 2020.01.28 10:01:41 Z

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME 6512	SAF/MEME 6512	MEME 697

DSO Signature



Digitally signed by David Green
Date: 2020.08.03 12:09:10 +01'00'
Adobe Acrobat version: 2020.009.20074

This document set must be reviewed and re-approved at the following times:

1) After the first occurrence of the activity described above (Review only)
2) After any change to the procedure or reagents used
3) After any incident resulting from this activity
4) At least annually from the date of approval

Next Review: 27/01/2021

Review comments