

## 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	Decellularisation of porcine tissues
Supervisor Name	Sotiris Korossis

### Risk Assessment

Reference

Location

Originator

Project / Activity / Task

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 cables current	Heat(Inc. IR)	+
Category 2: Workplace				
Slips/Trips/Falls on the level				+
Slips/Trips/Falls on the level				X
Category 3: Hazardous and/or Harmful substances				
Flammable substances				+
Flammable substances				X
Corrosive substances				X
Irritant substances				X
Category 4: Work activity				
Lone working out of hours				+
Lone working out of hours				X
Category 5: Work organisation				
N/A				+
N/A				X

### Explain the risks associated with these hazards

People / Groups at risk	<input type="text" value="Operator and people in proximity"/>			X
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Slips/Trips/Falls on the level"/>	<input type="text" value="Harmful"/>	<input type="text" value="Highly Unlikely"/>	Low	
What are the control measures?	Lowers Impact	Lowers Probability	+	
<input type="text" value="Organise room to have nothing on the floor that can be a trip hazard. Reduce movement between labs if possible."/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	X	
			Residual Risk	
			Low	
People / Groups at risk	<input type="text" value="Operator and people in proximity"/>			X
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Aerosols/splashes from irritant substances &amp; sensitiser"/>	<input type="text" value="Very Harmful"/>	<input type="text" value="Likely"/>	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	

## Process Risk Assessment Form (Continued)

Work in fume hood	Significantly	Significantly	x	
Wear nitrile gloves	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score	
Hydrochloric acid can cause exothermic reaction	Slightly Harmful	Highly Unlikely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Do not mix with amines, aldehydes, permanganates, for example potassium permanganate.	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Everyone in the room			x
Enter risk details here:-	Impact	Probability	Risk Score	
Ignition or formation of inflammable gases/vapours	Very Harmful	Likely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Do not mix hydrochloric acid with aluminium, carbides, fluorine, metals, bases, sulphides.	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Everyone in the room			x
Enter risk details here:-	Impact	Probability	Risk Score	
Explosion	Very Harmful	Likely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Do not mix hydrochloric acid with alkali metals, sulphuric acid.	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator and people in proximity			x
Enter risk details here:-	Impact	Probability	Risk Score	
Hydrogen release	Slightly Harmful	Highly Unlikely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Avoid contact of hydrochloric acid with metals.	None	None	x	
			Residual Risk	
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Corrosion	Harmful	Likely	High	

## Process Risk Assessment Form (Continued)

What are the control measures?	Lowers Impact	Lowers Probability	+	
Avoid contact of hydrochloric acid with metals.	Significantly	Significantly	x	
				Residual Risk Low
People / Groups at risk	Everyone in the room			x
Enter risk details here:-	Impact	Probability	Risk Score	
Toxic substances	Slightly Harmful	Highly Unlikely		
What are the control measures?	Lowers Impact	Lowers Probability	+	
Work in fume hood	Significantly	Significantly	x	
Wear protective equipment	Significantly	Significantly	x	
				Residual Risk Low
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Splashes from hydrochloric acid	Very Harmful	Likely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Use butyl-rubber gloves with minimum layer thickness: 0.7 mm, break through time: > 480 min. Preferably, KCL 898 Butoject®	Significantly	Significantly	x	
				Residual Risk Low
People / Groups at risk	Everyone in the room			x
Enter risk details here:-	Impact	Probability	Risk Score	
Flammable substances	Very Harmful	Likely	Unacceptable	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Do not use chemicals at high temperatures	Significantly	Significantly	x	
Remove sources of ignition	Significantly	Significantly	x	
				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

## Process Risk Assessment Form (Continued)

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
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
<b>Total</b>	<b>1</b>	<b>8</b>	<b>7</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>16</b>

With these controls in place, the risk is:

**The activity is LOW RISK - and is effectively controlled**

# Safety Method Statement

Reference SAF/MEME 6511

Location H25,H34

Originator Sotiria Toumpaniari

Project / Activity / Task Decellularisation of porcine tissues

## What equipment will be used in this activity?

	+
Orbital shaker	X
Minisart Syringe Filter	X
Duran bottles	X
Autoclave	X
Centrifuge tubes	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)

	+
Polymixin B sulphate salt powder	X
Vancomycin hydrochloride hydrate	X
Gentamycin sulphate	X
DPBS without calcium, magnesium x10	X
EDTA	X
SDS	X
Tris	X
Triton X-100	X
Sodium hydroxide	X
Hydrochloric acid (6N)	X
CASO bouillon	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

## Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
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
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.	Be careful not spilling solution and treat waste according to COSSH forms.	X
All solution changes should take place in a biological safety cabinete to ensure sample sterility.	Avoid spillages.	X
Stirring of samples can take place on a bench providing samples are in bottles.	Make sure the bottles are well closed.	X


### COSHH Form

Reference MEME 690,691,692,693,6


Location H25,H34

Originator Sotiria Toumpaniari

Project / Activity / Task Decellularisation of porcine tissues

<b>CHEMICAL NAME</b> <b>Vancomycin hydrochloride hydrate</b>						Hazard Rating <b>High</b>		<b>OVERALL RISK:</b> <b>Low</b>
CAS No. 1404-93-9	Amount used 0.25 ml	Period of use (hrs) 1	The process is: Semi Closed	Physical State: Non-Volatile Liquid	<input type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential <b>Low</b>		
W.E.L. (Itel / stel)	<p><b>This chemical has a high health risk associated with it.</b></p>							

Hazard Statement and Description	Precaution Statement and Description	
H317 May cause an allergic skin reaction.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	X
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
	P284 Wear respiratory protection.	X
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.	X
	P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/poison specialist.	X
Justify the use of this chemical:		
How will the precautions listed above be implemented?		
Use personal protective equipment (gloves and lab coat). Use chemical in BSC or fume hood. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.		
Special Storage and Containment Measures	Disposal Method	
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in freezer at -20 °C.	Aqueous waste - Collect in bottle and when full, place it in pod 2.	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. <a href="#">Click here to see spill procedures</a></i>	
Absorbent non-combustible material		


<b>CHEMICAL NAME</b> <b>Gentamicin sulfate</b>						Hazard Rating <b>High</b>		<b>OVERALL RISK:</b> <b>Low</b>
CAS No. 1405-41-0	Amount used 0.25 g	Period of use (hrs) 1	The process is: Semi Closed	Physical State: Lyophilised Solid	<input type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential <b>Low</b>		
W.E.L. (Itel / stel)	<p><b>This chemical has a high health risk associated with it.</b></p>							

Hazard Statement and Description	Precaution Statement and Description	
H317 May cause an allergic skin reaction.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X






COSHH Form (Continued)

Justify the use of this chemical:		
How will the precautions listed above be implemented?		
Use personal protective equipment (gloves and lab coat). Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Use the powder form of the chemical in BSC or fume hood. Do not let product enter drains.		
Special Storage and Containment Measures	Disposal Method	+
Store in the fridge at 2 - 8 °C. Keep container tightly closed in a dry and well-ventilated place.	Aqueous waste - Collect in bottle and when full, place it in pod 2.	x
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. <a href="#">Click here to see spill procedures</a></i>	
Absorbent cloth / tissue or spill kit for larger volumes		

<b>CHEMICAL NAME</b> <b>Ethylenediaminetetraacetic acid</b>			Hazard Rating <b>High</b>		<b>X</b>
CAS No. <input type="text" value="60-00-4"/>	Amount used <input type="text" value="0.5"/>	Period of use (hrs) <input type="text" value="g"/>	The process is: <input type="text" value="Semi Closed"/>	Physical State: <input type="text" value="Dusty Solid"/>	<b>OVERALL RISK:</b> <b>Medium</b>
W.E.L. (Itel / stel) <input type="text"/>				<input checked="" type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested Exposure Potential: <b>Low</b>	




<b>Hazard Statement and Description</b>	<b>Precaution Statement and Description</b>	+
H319 Causes serious eye irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	x
How will the precautions listed above be implemented?		
Use personal protective equipment (lab coat and goggles). Avoid contact with skin and eyes. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust. Do not let product enter drains.		
Special Storage and Containment Measures	Disposal Method	+
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	Dilute prepared solution to 1-10mM and pour down the drain.	x
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. <a href="#">Click here to see spill procedures</a></i>	
Spill kit		

<b>CHEMICAL NAME</b> <b>Sodium dodecyl sulfate</b>					Hazard Rating <b>High</b>	
CAS No. <input type="text" value="151-21-3"/>	Amount used <input type="text" value="5"/>	Period of use (hrs) <input type="text" value="g"/>	The process is: <input type="text" value="Semi Closed"/>	Physical State: <input type="text" value="Dusty Solid"/>	<b>OVERALL RISK:</b> <b>Medium</b>	
W.E.L. (Itel / stel) <input type="text"/>				<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested Exposure Potential: <b>Low</b>		


<b>Hazard Statement and Description</b>	<b>Precaution Statement and Description</b>	+
H228 Flammable solid.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	x
H302 Harmful if swallowed.	P273 Avoid release to the environment.	x
H315 Causes skin irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H318 Causes serious eye damage.	P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you	x
H335 May cause respiratory irritation.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x
H412 Harmful to aquatic life with long lasting effects.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	x

# COSHH Form (Continued)

	P310 Immediately call a POISON CENTER or doctor/physician.	X
How will the precautions listed above be implemented?		
Use personal protective equipment (lab coat and goggles). Avoid contact with skin and eyes. Avoid dust formation and breathing it. Ensure adequate ventilation. Use the powder form of the chemical in fume hood in H34. Do not let product enter drains.		
Special Storage and Containment Measures	Disposal Method	+
Keep the SDS solution in a tightly-closed container. Store in a dry, cool and well-ventilated place. Do not refrigerate, as the SDS will precipitate out of solution.	If in powder form, discard in yellow bag. If in solution, pour it in drain and add copious amounts of water.	X
How will spillages be dealt with?		
<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. <a href="#">Click here to see spill procedures</a></i>		
Pick up powder using a wet absorbent tissue to avoid creating dust. If in solution, use absorbent tissue.		

<b>CHEMICAL NAME</b>					X	
<b>Triton X-100</b>				Hazard Rating <b>High</b>	<b>OVERALL RISK:</b> <b>Medium</b>	
CAS No. 9036-19-5	Amount used	Period of use (hrs)	The process is:	Physical State		<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested
W.E.L. (Itel / stel)	5 ml	24	Semi Closed	Non-Volatile Liquid		

Hazard Statement and Description	Precaution Statement and Description	
H302 Harmful if swallowed.	P273 Avoid release to the environment.	X
H315 Causes skin irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H318 Causes serious eye damage.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
H410 Very toxic to aquatic life with long lasting effects.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
	P313 Get medical advice/attention.	X
How will the precautions listed above be implemented?		
Use personal protective equipment (lab coat and goggles). Do not breathe aerosols. Avoid substance contact with skin and eyes. If product enter drains, dilute it with plenty of water.		
Special Storage and Containment Measures	Disposal Method	+
Keep solution in a tightly-closed container. Store in a dry, cool and well-ventilated place.	The Triton X-100 can be discarded via drainage followed by copious amounts of water.	X
How will spillages be dealt with?		
<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. <a href="#">Click here to see spill procedures</a></i>		
Absorbent cloth / tissue		

<b>CHEMICAL NAME</b>					X	
<b>Sodium hydroxide</b>				Hazard Rating <b>High</b>	<b>OVERALL RISK:</b> <b>Medium</b>	
CAS No. 1310-73-2	Amount used	Period of use (hrs)	The process is:	Physical State		<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested
W.E.L. (Itel / stel)	1 ml		Semi Closed	Dense Solid		

Hazard Statement and Description	Precaution Statement and Description	
H290 May be corrosive to metals.	P260 Do not breathe dust/fume/gas/mist/vapours/spray.	X

COSHH Form (Continued)



H314 Causes severe skin burns and eye damage.	P260 Do not breathe dust/fume/gas/mist/vapours/spray.	X
	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated...	X
	P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for...	X
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov...	X

How will the precautions listed above be implemented?

Wear PPE- nitrile gloves, lab coat, closed shoes, goggles.

Special Storage and Containment Measures	Disposal Method	
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	Solution can be neutralised with hydrochloric acid and can be discarded in the drain. Pellets can be discarded in yellow bag.	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.</i> <a href="#">Click here to see spill procedures</a>	

Absorbent cloth / tissue

<b>CHEMICAL NAME</b>					 		Hazard Rating	<b>OVERALL RISK:</b>  <b>Medium</b>
Hydrochloric acid							High	
CAS No. 258148	Amount used	Period of use (hrs)	The process is:	Physical State	<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential	Low	
W.E.L. (Itel / stel)	0.5 ml		Semi Closed	Non-Volatile Liquid				

Hazard Statement and Description	Precaution Statement and Description	
H290 May be corrosive to metals.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H314 Causes severe skin burns and eye damage.	P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.	X
H318 Causes serious eye damage.	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated...	X
H335 May cause respiratory irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov...	X
	P310 Immediately call a POISON CENTER or doctor/physician.	X

How will the precautions listed above be implemented?

Wear PPE- nitrile gloves, lab coat, closed shoes, goggles. It can be used on the bench, but it is preferable to be used in fume hood in H34.  
 Avoid contact with the following:  
 Exothermic reaction with amines, aldehydes, permanganates, for example potassium permanganate.  
 Risk of ignition or formation of inflammable gases or vapours with: aluminium, carbides, fluorine, metals, bases, sulphides.  
 Risk of explosion with: alkali metals, sulphuric acid.  
 Gives off hydrogen by reaction with metals.  
 Corrosive to metals.

Special Storage and Containment Measures	Disposal Method	
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.	Solution can be neutralised using sodium hydroxide and then, can be poured in the drain.	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.</i> <a href="#">Click here to see spill procedures</a>	

Spill kit

+ Add another chemical

Statement of work (Process to be undertaken)

Preparation of solutions:

- PBS (11L total): Prepare 11L that are required for the preparation of disinfection solution, 12 washes, and 1 sterility check.
- Disinfection solution: Disinfection solution is made up by supplementing 500 ml of PBS with 0.1-1 mg·ml<sup>-1</sup> of gentamicin, 0.1-1 mg·ml<sup>-1</sup> of polymyxin B and 0.01-0.1 mg·ml<sup>-1</sup> of vancomycin hydrochloride. The pH is adjusted to 7.2 - 7.4, and the solution is sterilised by filtration using a Stericup filter unit with 0.22 µm pore size filter.
- Hypotonic buffer plus EDTA (2.7 mM, 10mM Tris): Prepare a stock solution of EDTA of 0.5M. Then, take 5.4ml from this solution and dissolve it in 900ml of distilled water, then add Tris (1.21g) to make the hypotonic buffer. The pH is adjusted to 8.0 – 8.2 and the volume made up to 1000ml. The solution is autoclaved at 121°C for 20 min and stored at room temperature for long term storage.
- SDS (0.1-1% w/v) in distilled water: Prepare 0.5% w/v solution, filter sterilise and store it for long term storage.
- Triton X-100 (0.1-1% w/v) in distilled water: Dissolve 5ml in 995ml of distilled water. Stir until dissolved. Filter sterilise it and then store it for up to 2 months roughly.
- CASO bouillon for sterility assessment: Prepare 5 x CASO bouillon stock solutions by adding 75g of CASO bouillon to 500ml of distilled water. The solution is autoclaved at 121°C for 20 min and stored at 4°C.

Show Image

Decellularisation procedure:

1st day (Monday):

1. Disinfection (37°C; 185 ± 5 rpm; 1h) (1ml x 1cm<sup>2</sup>)
2. Hypotonic buffer (RT; 185 ± 5 rpm; 0-12h) (NOTE: change the solution after 6 hours minimum!) (2ml x 1cm<sup>2</sup>)
3. Hypotonic buffer (RT; 185 ± 5 rpm; 12-24h) (NOTE: leave overnight, so to treat the sample in the solution for a total of at least 24h) (2ml x 1cm<sup>2</sup>)

2nd – 3rd day (Tuesday, Wednesday) (detergents (RT; 185 ± 5 rpm))

1. 0.5 % Triton X-100 (0-12h) (NOTE: change the solution after 6 hours minimum!) (2ml x 1cm<sup>2</sup>)
2. 0.5 % Triton X-100 (12-24h) (NOTE: leave overnight, so to treat the sample in the solution for a total of at least 24h) (2ml x 1cm<sup>2</sup>)
3. 0.5 % SDS (0-12h) (NOTE: change the solution after 6 hours minimum!) (2ml x 1cm<sup>2</sup>)
4. 0.5 % SDS (12-24h) (NOTE: leave overnight, so to treat the sample in the solution for a total of at least 24h) (2ml x 1cm<sup>2</sup>)

4th – 10th day (Thursday-Friday) (rinsing (RT; 185 ± 5 rpm)) (2ml x 1cm<sup>2</sup> for all the washes)

1. PBS 1x (0-12h) (NOTE: change the solution after 6 hours minimum, then change after overnight step! Leave samples in PBS during the weekend and count the wash as a 12 hours wash. Do a total of 12 washes!) (Thursday).
2. PBS 1x (12-24h) (Thursday)
3. PBS 1x (24-36h) (Friday)
4. PBS 1x (36-48h) (Friday)
5. PBS 1x (48-60h) (Monday)
6. PBS 1x (60-72h) (Monday)
7. PBS 1x (72-84h) (Tuesday)
8. PBS 1x (84-96h) (Tuesday)
9. PBS 1x (96-108h) (Wednesday)
10. PBS 1x (108-120h) (Wednesday)
11. PBS 1x (120-132h) (Thursday)
12. PBS 1x (132-144) (Thursday)
13. Sterility check is performed as followed: cut a small piece of pericardium and put it in the soy casein medium. Assess sterility, in terms of medium turbidity, after 14 days incubation (Friday).
14. Storage in falcon tubes in sterile PBS with 1% v/v P/S, (30ml PBS + 300µl P/S, each sample) at 4° C for up to 3 months (Friday).

Personal protection requirements not covered in the precaution statements above.

Closed shoes, over shoes (required for work in CBE)

Sources of information and references

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=P4932&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fp4932%3Flang%3Den>

Reference to **existing approved** Risk Assessment

## COSHH Form (Continued)

<https://ehs.ucsf.edu/chemicals-approved-drain-disposal#E>  
<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=L6026&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2F6026%3Fflang%3Den>  
<http://www.ncbe.reading.ac.uk/SAFETY/SDS/SodiumDodecylSulphate.pdf>  
[https://www.merckmillipore.com/GB/en/product/msds/MDA\\_CHEM-108603?Origin=PDP](https://www.merckmillipore.com/GB/en/product/msds/MDA_CHEM-108603?Origin=PDP)  
<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=795429&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2F795429%3Fflang%3Den>  
<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=258148&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2F258148%3Fflang%3Den>  
<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=258148&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2F258148%3Fflang%3Den>

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

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

### Form Reference Numbers

Risk Assessment

SAF/MEME 6511

Method Statement

SAF/MEME 6511

COSHH Assessment

MEME 690,691,692,693,6

DSO Signature

### **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