

## Safety Documentation

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

**Method Statement**

**Risk Assessment**

**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	Eleanor Knight
email address	e.knight@lboro.ac.uk
Location	Garendon Wing, Holywell park
Project / Activity / Task	RNA extraction and qPCR
Supervisor Name	Carmen Torres-Sanchez

# Safety Method Statement

Reference SAF/MEME/7755

Location Garendon Wing, Holywell park

Originator Eleanor Knight

Project / Activity / Task RNA extraction and qPCR

What equipment will be used in this activity?	+
Eppendorf 5804 centrifuge	X
Sartorius-Stedim Centrisart A-14 Microcentrifuge	X
Applied Biosystems StepOne Real-Time PCR system	X
Applied Biosystems 96 well fast thermal cycler	X

What training must be completed to do this activity?	+
CBE induction and completion of safety training record	X

What chemicals are being used? (These must be included in the COSHH Form)	+
Qiagen RNeasy mini kit Buffer RLT	X
Qiagen RNeasy mini kit Buffer RW1	X
RNase Zap	X
Quantinova SYBR Green RT-PCR kit	X
2-mercaptoethanol	X
PureLink RNA mini kit	X

Spill and accident procedures.	+
Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable closed container for disposal. As detailed in SOP039.	X

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
If working with 2-mercaptoethanol - Seal container and leave in the fume hood, with the extraction turned on. Label clearly any working solutions that have been used. All other solutions are at such low concentrations that they are non-hazardous.	X

References.	+
SAF/MEME/6684 Use of the Applied Biosystems StepOne Real-Time PCR system and the Applied Biosystems 96 well fast thermal cycler.	X
SOP039 Storage, handling and disposal of waste chemicals	X
Qiagen RNeasy Mini Handbook	X
PureLink RNA Mini Kit Users guide	X
CBE BRA147 Biocompatibility of 3D printed Ti scaffolds: A systematic study of in-vitro cytotoxicity and osteoinductive properties of 3D printed titanium scaffolds with different porous architectures	X

## Detailed sequential description of the process

Process step	Precautionary measures and comments	+

## Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
1. Prepare lysis buffer by adding 10ul/ml 2-mercaptoethanol	Work is to be carried out under the chemical fume hood. Wear suitable PPE as required in CBE labs + safety spectacles. Dispose the tips in the purple plastic box for cytotoxic hazardous materials and seal it. Avoid spillages. Only makeup required amount of lysis buffer.	X
2. Prior to cell lysis, Cells cultured on scaffolds are set in a 24/48 multi-well plate. Remove cell culture media and add lysis + BME buffer from chosen kit (either RNAeasy or Purelink)	Cell culture is done under precautions set out in CBEBRA147. Samples should be processed in a biological safety cabinet until samples are fully lysed. Throughout the protocol suitable PPE should be worn as required in the CBE labs - this includes labcoat, gloves, shoe covers	X
3. Homogenise samples using 21G sterile needle	Precautions should be taken when using sharps - needles should never be resheathed. Needles should be dispose of in yellow sharps bins with purple lids. Throughout the protocol suitable PPE should be worn as required in the CBE labs - this includes labcoat, gloves, shoe covers	X
4. Continue with RNA extraction using protocols detailed in either: a) Rneasy Mini handbook - Purification of Total RNA using Spin Technology b) Purelink RNA MiniKit user guide - Purifying RNA from Animal and Plant cells	Buffers for RNA extraction contain guanidine thiocyanate, guanidine hydrochloride, and a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. Throughout the protocol suitable PPE should be worn as required in the CBE labs - this includes labcoat, gloves, shoe covers	X
5. Continue with RT PCR reaction using QuantiNova SYBR Green one step RT PCR kit	All components of this kit are considered non- hazardous. The use of the Applied Biosystems StepOne Real-Time PCR system and the Applied Biosystems 96 well fast thermal cycler, have already been considered in SAF/ MEME/6684 and is LOW RISK	X
		X
		X
		X
		X
		X
		X
		X

# Risk Assessment

Reference

Location

Originator

Project / Activity / Task

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

Category 1: Machinery & work equipment:				
Design and Construction	Mechanical hazards	Electrical hazards	Radiation hazards	
N/A	N/A	Electrical test lables current	N/A	+
Category 2: Workplace				
N/A				+
Category 3: Hazardous and/or Harmful substances				
Irritant substances				+
Harmful if swallowed				x
Cause serious eye damage				x
Suspected of damaging the unborn child				x
Very toxic to aquatic life				x
Category 4: Work activity				
Use of needle sharps				+
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 only"/>			x
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Irritant substances"/>	<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="Wear suitable PPE, Lab coat, gloves, safety glasses"/>	<input type="text" value="Significantly"/>	<input type="text" value="Slightly"/>	x	
			Residual Risk	
			<input type="text" value="Low"/>	
People / Groups at risk	<input type="text" value="Operator only"/>			x

## Process Risk Assessment Form (Continued)

Enter risk details here:- Harmful if swallowed	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Wear suitable PPE, Lab coat, gloves, safety glasses. Follow normal lab rules, no eating and drinking etc.	Significantly	Moderately	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- causes serious eye damage	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Wear suitable PPE, Lab coat, gloves, including safety glasses	Significantly	Slightly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Suspected of damaging the unborn child	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Expectant mothers should not use 2-mercaptoethanol, ask another trained lab member to assist with this part of protocol. All other users to wear suitable PPE, Lab coat, gloves, including safety glasses and work in a fume hood. contaminated pipette tips should be dispose off in the yellow bins with purple lids	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Very toxic to aquatic life	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
None of the buffers contained 2-mercaptoethanol should enter the drainage system. 2-mercaptoethanol to be used in fume hoods and contaminated pipette tips will be dispose of in yellow bins with purple lids	Moderately	Moderately	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Use of needle sharps	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+

## Process Risk Assessment Form (Continued)

Precautions should be taken when using sharps - needles should never be resheathed. Needles should be disposed of in yellow sharps bins with purple lids.	Significantly	Moderately	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	0	0	0	0	0	0	0
Technical Staff	0	1	0	0	0	0	1
Research Staff (PDRA)	2	0	0	0	0	0	2
Research Students (PhD)	0	0	0	0	0	1	1
Students (Undergraduate / MSc)	0	0	0	0	0	1	1
Visitors	0	0	0	0	0	1	1
Others - Over-type as needed	0	0	0	0	0	1	1
<b>Total</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>7</b>

With these controls in place, the risk is:

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


### COSHH Form

Reference SAF/MEME/2066 - 2072

Location Garendon Wing, Holywell park


Originator Eleanor Knight

Project / Activity / Task RNA extraction and qPCR

<b>CHEMICAL NAME</b> 2-mercaptoethanol						Hazard Rating High	<b>OVERALL RISK:</b> Medium
CAS No. 60-24-2	W.E.L. (Itel / stel)	Amount used 0.01 ml	Period of use (hrs) 3	The process is: Semi Closed	Physical State Non-Volatile Liquid	Exposure Potential Low	

This chemical has a high health risk associated with it.



Hazard Statement and Description	Precaution Statement and Description	
H310 Fatal in contact with skin.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H317 May cause an allergic skin reaction.	P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.	X
H318 Causes serious eye damage.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
H361d Suspected of damaging the unborn child.	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	X
H301 + H307 Toxic if swallowed or if inhaled.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	X
Justify the use of this chemical:	2-mercaptoethanol is required to deactivate RNAses, there is no suitable alternative. Very small amounts of the chemical are required 10ul/ml. Expectant mothers will not carry out this step of the protocol	
How will the precautions listed above be implemented?		
Expectant mothers will not carry out this step of the protocol. Wear the correct PPE. Ensure adequate ventilation. Work under the Chemical Fume hood. Use small aliquots. Avoid spillages. Keep the work surface clean and organized.		
Special Storage and Containment Measures	Disposal Method	
Tightly closed. Keep in a well-ventilated place. Keep locked up or in an area accessible only to qualified or authorized persons. Tightly closed. Keep in a well-ventilated place. Keep locked up or in an area accessible only to qualified or authorized persons	Other - Check with Technician / Supervisor dispose of as chemical waste.	X
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. <a href="#">Click here to see spill procedures</a>	
Spill kit		

<b>CHEMICAL NAME</b> Buffer RLТ						Hazard Rating High	<b>OVERALL RISK:</b> Medium
CAS No.	W.E.L. (Itel / stel)	Amount used 3 ml	Period of use (hrs) 30	The process is: Semi Closed	Physical State Non-Volatile Liquid	Exposure Potential Low	


Hazard Statement and Description	Precaution Statement and Description	
H302 Harmful if swallowed.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X

COSHH Form (Continued)

H318 Causes serious eye damage.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
How will the precautions listed above be implemented?		
Wear the correct PPE - lab coat, gloves, safety glasses		
Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place.	The product should not be allowed to enter drains, water courses or the soil. Dispose of as chemical waste	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>	
If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite		

<b>CHEMICAL NAME</b> <b>Buffer RW1</b>			Hazard Rating <b>High</b>	<b>OVERALL RISK:</b> <b>Low</b>
CAS No. <input style="width: 100%;" type="text"/>	Amount used <input type="text" value="8"/> <input type="text" value="ml"/>	Period of use (hrs) <input type="text" value="1"/>	The process is: <input type="text" value="Semi Closed"/> Physical State: <input type="text" value="Volatile Liquid"/>	
W.E.L. (Itel / stel) <input style="width: 100%;" type="text"/>	<input checked="" type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		Exposure Potential <b>Low</b>	

Hazard Statement and Description	Precaution Statement and Description	+
H226 Flammable liquid and vapour.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	X
H318 Causes serious eye damage.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
<input style="width: 100%;" type="text"/>	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
<input style="width: 100%;" type="text"/>	P310 Immediately call a POISON CENTER or doctor/physician.	X
How will the precautions listed above be implemented?		
Wear the correct PPE - lab coat, gloves, safety glasses		
Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place. Do not store together with oxidizing and self-igniting products.	Hydrophylic organic solvent waste	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>	
If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite		

<b>CHEMICAL NAME</b> <b>Wash Buffer 1</b>		Hazard Rating <b>High</b>	<b>OVERALL RISK:</b> <b>Low</b>
CAS No. <input style="width: 100%;" type="text"/>	Amount used <input type="text" value="50"/> <input type="text" value="ml"/>	Period of use (hrs) <input type="text" value="1"/>	
W.E.L. (Itel / stel) <input style="width: 100%;" type="text"/>	<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		Exposure Potential <b>Low</b>

Hazard Statement and Description	Precaution Statement and Description	+
H315 Causes skin irritation.	P264 Wash hands thoroughly after handling.	X
H319 Causes serious eye irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X



COSHH Form (Continued)

	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
	P332 + P313 If skin irritation occurs: Get medical advice/attention.	X
	P337 + P313 If eye irritation persists: Get medical advice/attention.	X
	P362 Take off contaminated clothing and wash before reuse.	X


How will the precautions listed above be implemented?

Wear the correct PPE - lab coat, gloves, safety glasses

Special Storage and Containment Measures	Disposal Method	
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers	The generation of waste should be avoided or minimized wherever possible. Empty containers may retain some product residues. This material and its container must be disposed of as chemical waste	X

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](#)*

If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite

<b>CHEMICAL NAME</b> Ethanol		Hazard Rating Medium	OVERALL RISK: <b>Low</b>
CAS No. 64-17-5 W.E.L. (Itel / stel)	Amount used: 50 ml Period of use (hrs): 1 The process is: Semi Closed Physical State: Volatile Liquid	Exposure Potential Low	

Hazard Statement and Description	Precaution Statement and Description	
H226 Flammable liquid and vapour.	P233 Keep container tightly closed.	X

How will the precautions listed above be implemented?

Very small amounts of ethanol are required less than 125ml. Chemical will be handled in fume hood and stock will be stored in suitable flammables cupboard

Special Storage and Containment Measures	Disposal Method	
In flammables cupboard	Aqueous waste - very small amounts can be diluted in water	X

<b>CHEMICAL NAME</b> Quantinova SYBR Green RT-PCR kit		Hazard Rating Low	OVERALL RISK: <b>Low</b>
CAS No. W.E.L. (Itel / stel)	Amount used: g Period of use (hrs): The process is: Semi Closed Physical State: Non-Volatile Liquid	Exposure Potential Low	

Hazard Statement and Description	Precaution Statement and Description	
No Hazard Statements applicable	No Precaution statements applicable	X

How will the precautions listed above be implemented?

# COSHH Form (Continued)

Special Storage and Containment Measures	Disposal Method	+
		x
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. <a href="#">Click here to see spill procedures</a>	

<b>CHEMICAL NAME</b>		Hazard Rating		<b>OVERALL RISK:</b> <b>Low</b>
RnaseZap		Low		
CAS No.	Amount used	Period of use (hrs)	The process is:	Physical State
W.E.L. (l/ tel / stel)	<input type="text"/> g	<input type="text"/>	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	<input type="checkbox"/> Non-Volatile Liquid
				Exposure Potential
				Low

Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	x
How will the precautions listed above be implemented?		
Special Storage and Containment Measures	Disposal Method	+
		x
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. <a href="#">Click here to see spill procedures</a>	

+ Add another chemical

Statement of work (Process to be undertaken)

Show Image

Personal protection requirements not covered in the precaution statements above.

Sources of information and references

MSDS from:  
Qiagen  
a) <https://www.qiagen.com/us/knowledge-and-support/product-and-technical-support/quality-and-safety-data/sds-search?l=US&q=800000000208>  
b) <https://www.qiagen.com/us/knowledge-and-support/product-and-technical-support/quality-and-safety-data/sds-search?l=US&q=800000005356>  
  
Sigma Aldrich  
c) <https://www.sigmaaldrich.com/GB/en/sds/aldrich/m6250>  
d) <https://www.sigmaaldrich.com/GB/en/product/mm/108543>  
e) <https://www.sigmaaldrich.com/GB/en/product/sigma/r2020>  
  
Thermofisher  
f) <https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2F>

Reference to **existing approved** Risk Assessment

## COSHH Form (Continued)

2FSDS%2F100015335\_MTR-EULT\_BE.pdf

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/7755

Method Statement

SAF/MEME/7755

COSHH Assessment

SAF/MEME/2066 - 2072

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:

11 Oct 2024

Review comments