

## **Safety Documentation**

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

<b>✓</b>	Risk Assessment	✓ Method Statement	
▼		V medica statement	<u>V</u>

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

**<u>Buttons</u>**: [+] 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 <u>MUST NOT</u> START ANY PRACTICAL WORK UNTIL THESE FORMS HAVE BEEN RETURNED TO YOU WITH **BOTH** YOUR SUPERVISOR'S AND DSO'S APPROVAL SIGNATURES ATTACHED.

Please comple	ete these fields
School or Service	Wolfson School of Mechanical, Electrical and Manufacturing Engineering
Department	Centre for Biological Engineering
Originator name	Maria Pavlidou; Sotiria Toumpaniari
email address	m.pavlidou@lboro.ac.uk; s.toumpaniari@lboro.ac.uk
Location	H27, H29, H30
Project / Activity / 1	ask Live/Dead staining
Supervisor Name	Prof. Sotiris Korossis

Version: 2.20

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## Risk Assessment

Reference	SAF/MEME/6807

Location	H27, F	129, H30		Originator	Maria Pavlidou; Sotiria Toumpa	aniari
Project / Activity / Task Live/Dead staining						
Is this process risk as	sessn	ment for a: 📿 Laboratory	/ Workshop	○ General us	е	
Category 1: Machiner	y & w	ork equipment:				
Design and Constructi	on	Mechanical hazards	Electrical	hazards	Radiation hazards	+
N/A		N/A	Electrical test la	bles current		x
Category 2: Workplac	e					+
Slips/Trips/Falls on the lev	/el					X
Category 3: Hazardou	s and	l/or Harmful substances				+
Suspected cancer causing substances						X
exposure to Covid-19						X
Category 4: Work acti	vity					+
Lone working out of hour	S					X

Explain the risks associated with these hazards					
People / Groups at risk Operator only			x		
Enter risk details here:-	Impact	Probability	Risk Score		
Electrical shock from using BSC and microscope	Harmful	Highly Unlikely	Low		
What are the control measures?	Lowers Impact	Lowers Probability	+		
Equipment has bi-annual PAT testing and visual checking of cables and connectors prior to use Operator trained in the use of equipment	Significantly	x			
			Residual Risk		
			Low		
People / Groups at risk Operator and people in proximity	X				
Enter risk details here:-	Risk Score				
Exposure of suspected cancer causing substance	Very Harmful	Unlikely	High		
What are the control measures?	Lowers Impact	Lowers Probability	+		

Category 5: Work organisation

N/A

#### Process Risk Assessment Form (Continued)

Operator will be aware of h accordingly and adhere to statement)	will be done for each hazardous chemical entified. hazards and will use substances CBE SOP (see references - Method appropriate PPE as identified in the risk	Significantly Significantly	Significantly	x	
Use reagent in biological sa	afety cabinet.	Significantly	Significantly	x	
					dual Risk _ow
People / Groups at risk Op	perator and people in proximity				x
Enter risk details here:-		Impact	Probability	Risk So	core
Slips trips and falls		Slightly Harmful	Highly Unlikely		
What are the control measures?		Lowers Impact	Lowers Probability	+	
Laboratory users are trained including dealing with spill	d on how to work with chemicals safely s.	None	None	x	
				Resid	dual Risk
People / Groups at risk Op	perator and people in proximity				x
Enter risk details here:-		Impact	Probability Risk Score		core
Exposure to Covid-19		Very Harmful	Highly Unlikely	Me	edium
What are the control measures?		Lowers Impact	Lowers Probability	+	
respect local Lab rules. Frequent washing (20 seco carried out. Distancing should be 2 met	d University Covid-19 guidelines, and nds minimum)/ sanitizing of hands to be tre, but 1M+ is allowed where all e coverings and this cannot be avoided g	None	Moderately	x	
	Residual Risk				

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

Personnel Group	Maximum (Task setup/ Reconfiguration)	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)	0	1	0	0	0	0	1
Research Students (PhD)	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
Students (Undergraduate / MSc)	0	0	0	0	0	0	0
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0
Total	0	3	0	0	0	0	3

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



SAF/MEME/6807 Reference Maria Pavlidou; Sotiria Toumpaniari Location H27, H29, H30 Originator Project / Activity / Task | Live/Dead staining What equipment will be used in this activity? + Fluorescent microscope (Nikon Eclipse Ti Series) X **Pipettes** Pipette tips X 24 well plates X **Biological Safety Cabinet** X What training must be completed to do this activity? + Cell culture X Aseptic technique Use of fluorescent microscope What chemicals are being used? (These must be included in the COSHH Form) Calcein AM in anhydrous DMSO X Ethidium homodimer-1(2mM solution) in DMSO/H2O 1:4 (v/v) X Dulbecco's Phosphate-Buffered Saline (DPBS) X X Spill and accident procedures. + In case of contact with eyes, remove contact lenses, if present and easy to do and immediately flush eyes with copious amounts of water for several minutes. In case of contact with skin, immediately wash skin with soap and copious amounts of water. Absorb the spillage using clean paper towels and dispose of the paper towels in a cytotoxic waste bag. Place the used towels and gloves in the cytotoxic waste bag. Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event) Leave note with a name of the operator and date mentioning not to move anything from the area and dispose contaminated gloves. If alarm sounds evacuate area References. CBE code of practice, SOP009, SOP072, SOPSOP037, SOP038, SOP039

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Detailed sequential description of the process

Process step

+

Precautionary measures and comments

## Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
In a BSC, prepare 10 mL of an approximately 2 $\mu$ M calcein AM and 4 $\mu$ M EthD-1 solution. More specifically, add 20 $\mu$ L of the supplied 2 mM EthD-1 stock solution to 10 mL of sterile, tissue culture–grade DPBS and 5 $\mu$ L of the supplied 4 mM calcein AM stock solution to the 10 mL EthD-1 solution.	Wear nitrile gloves and a lab coat.	x
In a BSC, transfer well plates with samples and wash with PBS. Aspirate PBS after wash.	Use aspirator.	x
Add working solution to cover the cell culture samples.	Cover samples with aluminum foil as soon as the working solution is added.	x
Incubate in the dark at RT for 20-25 min	Leave the samples that have been covered with aluminum foil on th bench and leave a note with the operator name, date and assay, so they will not be discarded.	x
Observe well plates under fluorescent microscope.	Work in the dark to protect the fluorescent dyes from bleaching.	x



#### **COSHH Form**

Reference

SAF/MEME/1040

Location H27, H29, H30 Maria Pavlidou; Sotiria Toumpaniari Originator

Project / Activity / Task | Live/Dead staining

		<del>,</del>				
CHEMICAL NAME  Dimethyl sulfoxide (95-100% w/v)/ 5,5-		Rating High OVERAL	X L			
CAS No. 67-68-5/61926-22-5 W.E.L. (Itel / stel)	Amount used Period of use (hrs)  0.2 ml 1	The process is: Physical State				
This chemical has a high health risk asso	ciated with it.					
Hazard Statement a	nd Description	Precaution Statement and Description	+			
H351 Suspected of causing cancer.		P201 Obtain special instructions before use.	x			
		P280 Wear protective gloves/protective clothing/eye protection/face protection.	x			
		P202 Do not handle until all safety precautions have been read and understood.				
		P308 + P313 IF exposed or concerned: Get medical advice/attention.				
		P501 Dispose of contents/container to				
Justify the use of this chemical:		Ethidium homodimer-1 is a membrane permeable fluorescent dye that helps identification of dead cells. Dead cells stained with Ethidium homodimer-1 are observed with red color under a fluorescent microscope. Therefore, dead cells can be distinguished from living cells, which will be stained with Calcein AM (green fluorescent dye).				
How will the precautions listed	l above be implemented?					
Wear PPE (lab coat, nitrile glove	es, goggles, shoe covers, lo	ong trousers and closed shoes). Treat waste as cytotoxic.				
Special Storage and Containment Measures		Disposal Method	+			
Store at ≤–20°C and protect from light.		Collection in waste bottle for Live/Dead staining and when it is full, it is going to be discarded as cytotoxic waste in pod 1.	x			
How will spillages be dealt wit	th?	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 and then, discard in cytotoxic waste.						
+ Add another chemical						

Statement of work (Process to be undertaken)

Ethidium homodimer-1 is a membrane permeable fluorescent dye which binds to DNA. Therefore, Ethidium homodimer-1 is used to stain dead cells which are observed with red color under a fluorescence microscope

**Show Image** 

Personal protection requirements not covered in the precaution statements above.

Long trousers, closed shoes, lab coat, nitrile gloves and overshoes.

Sources of information and references

https://www.thermofisher.com/order/catalog/product/L3224#/L3224

Reference to existing approve	<u>d</u> Risk Assessment

#### **COSHH Form (Continued)**

https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FSDS%2FL3224COMPONENTA\_MTR-EULT\_BE.pdf&title=TDMyMjRDT01QT05FTIRB

https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FSDS%2FL3224COMPONENTB\_MTR-EULT\_BE.pdf&title=TDMyMjRDT01QT05FTIRC

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, Not Approved 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. **Supervisors Signature** Form Reference Numbers Method Statement **COSHH Assessment** Risk Assessment SAF/MEME/6807 SAF/MEME/1040 SAF/MEME/6807 **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)

<ul><li>2) After any change to the procedure or reagents used</li><li>3) After any incident resulting from this activity</li><li>4) At least annually from the date of approval</li></ul>	Next Review:	21 May 2022
Review comments		