

## 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    [- ] 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	School of Aeronautical, Automotive, Chemical and Materials Engineering
Department	Department of Chemical Engineering
Originator name	Nishant Joglekar
email address	n.joglekar@lboro.ac.uk
Location	Centre for Biological Engineering (CBE)
Project / Activity / Task	Caspase 3/7 Apoptosis Assay
Supervisor Name	Karen Coopman and Elizabeth Ratcliffe

# 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	N/A	+
Category 2: Workplace				
Slips/Trips/Falls on the level				+
Category 3: Hazardous and/or Harmful substances				
Irritant substances - DMSO and ethanol are irritants; refer to COSHH forms below				+
Cancer causing substances - fixative may cause cancer if exposed; refer to COSHH forms below				+
Category 4: Work activity				
N/A				+
Category 5: Work organisation				
N/A				+

Explain the risks associated with these hazards				
People / Groups at risk	<input type="text" value="Operator only"/>			+
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Exposure to hazardous substances for operator"/>	<input type="text" value="Harmful"/>	<input type="text" value="Unlikely"/>	Medium	
What are the control measures?	Lowers Impact	Lowers Probability	+	
<input type="text" value="Appropriate PPE will be worn"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
<input type="text" value="Spillages will be dealt with immediately as per risk assessment"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
<input type="text" value="Work will be done in a BSC"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
<input type="text" value="Only small amounts of reagents will be used - see COSHH forms"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
			Residual Risk	
			<input type="text" value="Low"/>	
People / Groups at risk	<input type="text" value="Everyone in the room"/>			+

## Process Risk Assessment Form (Continued)

Enter risk details here:- Exposure to hazardous substances for others in the labs	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
In H30, where the NucleoCounter is situated, only one person is allowed at a time due to social distancing measures	Significantly	Significantly	x
Work in H23 will be performed inside a BSC limiting the chance of exposure to others in the room	Significantly	Significantly	x
All lab users will be wearing appropriate PPE as per risk assessments limiting the chance of exposure	Significantly	Significantly	x
Spillages will be dealt with immediately as per risk assessment	Significantly	Significantly	x
Only small amounts of reagents will be used - see COSHH forms	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Everyone in the room		x
Enter risk details here:- Risk of fire due to ethanol	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Work with ethanol will be done in a fume hood/BSC	Significantly	Significantly	x
There will not be any sources of ignition near the ethanol	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator and people in proximity		x
Enter risk details here:- Slips/Trips/Falls on the level	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
No running in the work area, Good House keeping ensuring floors and surfaces are clear of clutter.	Moderately	Moderately	x
Ensure spills are cleared up immediately after occurring	Slightly	Slightly	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	0	0	1	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 Staff (PDRA)	0	0	0	1	0	0	1
Research Students (PhD)	0	1	0	1	0	0	2
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
<b>Total</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>4</b>

With these controls in place, the risk is:

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

# Loughborough University

## Department of Chemical Engineering

### Safety Method Statement

Reference SAF/MEME 6530

Location Centre for Biological Engineering (CBE) Originator Nishant Joglekar

Project / Activity / Task Caspase 3/7 Apoptosis Assay

What equipment will be used in this activity?	+
Nucleocounter	X
BSC	X
Centrifuge	X
Flow cytometer	X
Water bath	X
Vortex	X

What training must be completed to do this activity?	+
Cell culture	X
Aseptic techniques	X
CBE induction	X
NucleoCounter	X
Flow cytometer	X

What chemicals are being used? (These must be included in the COSHH Form)	+
FAM-DEVD-FLICA, Caspase-3/7 inhibitor FLICA reagent (part of kit)	X
Propidium Iodide (ready-made solution part of kit)	X
Hoechst 33342 (ready-made solution part of kit)	X
Fixative (mixture part of kit)	X
DMSO	X
Ethanol	X
Staurosporine solution	X

Spill and accident procedures.	+
Spillages are likely to be less than 1ml and inside a BSC. Spillages such as these can be cleaned up with an absorbent cloth/tissue using 1:20 Chemgene. Specific disposal procedures must be followed depending on the chemicals involved in the spillage, with tissues containing non-hazardous chemical spills going down the yellow stream waste, and tissues containing hazardous chemical spills being disposed as cytotoxic chemical waste in purple and yellow waste bags.	X
In the unlikely case of a small but significant spillage (still less than 10ml) resulting from a bottle containing a chemical being knocked over i.e. DMSO, people in immediate area of spill will be alerted, the spill area will be covered with paper towels soaked with 1% Virkon solution and left for 10 minutes. The soaked paper towels (and other virkon soaked items) will then be put into a yellow biohazard disposal bag. Lab staff will be informed when clean-up is complete and spill record in the logbook will be filled. A larger spillage (greater than 10ml) is not likely to occur.	X

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event) +

## Safety Method Statement (Continued)

Remove contaminated PPE or clothing. Alert other laboratory staff and leave the laboratory immediately while leaving the BSC switched on and leaving any cultures inside the cabinet. Wash hands and other potentially contaminated areas with soap and water.	X
Make sure that all the containers are tightly closed and stored upright in a well-ventilated place.	X
Close laboratory doors and post warning signs to prevent others entering the laboratory and report the incident to the Laboratory Manager.	X

### References.

	+
SOP039	X
SOP038	X
<a href="https://immunochemistry.com/wp-content/uploads/2016/05/93-94-FAMDEVD-KIT-SDS-2.pdf">https://immunochemistry.com/wp-content/uploads/2016/05/93-94-FAMDEVD-KIT-SDS-2.pdf</a>	X
<a href="https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&amp;language=en&amp;productNumber=56942&amp;brand=SIGMA&amp;PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fs6942%3Flang%3Den">https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&amp;language=en&amp;productNumber=56942&amp;brand=SIGMA&amp;PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fs6942%3Flang%3Den</a>	X
<a href="https://store.apolloscientific.co.uk/storage/msds/BID1200_msds.pdf">https://store.apolloscientific.co.uk/storage/msds/BID1200_msds.pdf</a>	X
<a href="https://immunochemistry.com/wp-content/uploads/2016/05/F18-91-6-D-1.pdf">https://immunochemistry.com/wp-content/uploads/2016/05/F18-91-6-D-1.pdf</a>	X
<a href="https://marketing.chemometec.com/acton/attachment/21287/f-00f0/1/-/-/-/994-3021-FLICA-Caspase-Assay.pdf">https://marketing.chemometec.com/acton/attachment/21287/f-00f0/1/-/-/-/994-3021-FLICA-Caspase-Assay.pdf</a>	X
SAF/289 - Reference of existing approved ethanol risk assessment	X
<a href="https://www.carlroth.com/medias/SDB-CN74-MT-EN.pdf?context=bWFzdGVyfHNIY3VyaXR5RGF0YXNoZWV0c3wyMDAwNjZ8YXBwbGljYXRpb24vcGRmfHNIY3VyaXR5RGF0YXNoZWV0cy9oOTYvaDI0Lzg5Njk2MDEyODYxNzQucGRmfDMYNTQ3OGU4M2M0MzcxMzUzNjYwZGU2OTZkMWM4NmIxYWZiMjJjODRmNDY1MTM0MzJmYTkWNTA1NTg4ZDIwYTk">https://www.carlroth.com/medias/SDB-CN74-MT-EN.pdf?context=bWFzdGVyfHNIY3VyaXR5RGF0YXNoZWV0c3wyMDAwNjZ8YXBwbGljYXRpb24vcGRmfHNIY3VyaXR5RGF0YXNoZWV0cy9oOTYvaDI0Lzg5Njk2MDEyODYxNzQucGRmfDMYNTQ3OGU4M2M0MzcxMzUzNjYwZGU2OTZkMWM4NmIxYWZiMjJjODRmNDY1MTM0MzJmYTkWNTA1NTg4ZDIwYTk</a>	X
<a href="https://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB_ENG_1351304.pdf">https://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB_ENG_1351304.pdf</a>	X

### Detailed sequential description of the process

Process step	Precautionary measures and comments	+
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## Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
<p>The following samples will be prepared:</p> <ol style="list-style-type: none"> <li>1) A positive FLICA control in which apoptosis has been induced in the cells using staurosporine prior to staining with just FLICA</li> <li>2) A negative FLICA control in which healthy cells are stained with just FLICA</li> <li>3) A positive Hoechst 33342 control in which apoptosis has been induced in the cells using staurosporine prior to staining with just Hoechst 33342</li> <li>4) A negative Hoechst 33342 control in which healthy cells are stained with just Hoechst 33342</li> <li>5) A positive FLICA and Hoechst 33342 control in which apoptosis has been induced in the cells using staurosporine prior to staining with both FLICA and Hoechst 33342</li> <li>6) A negative FLICA and Hoechst 33342 control in which healthy cells are stained with both FLICA and Hoechst 33342</li> <li>7) An unlabeled positive control in which apoptosis has been induced in the cells using staurosporine and no staining has been performed where DMSO has been used as a vehicle</li> <li>8) An unlabelled negative control in which cells are healthy and no staining has been performed where DMSO has been used as a vehicle</li> <li>9) A positive propidium iodide (PI) control in which the cells have been killed using ethanol prior to staining with just PI</li> <li>10) A negative PI control in which healthy cells are stained with just PI</li> <li>11) Test sample which will involve staining with FLICA, Hoechst 33342 and PI.</li> </ol> <p>100,000 cells will be used for each sample. The ten controls listed above will be run prior to the main experiment for background corrections.</p> <p>On the day of the experiment, along with the test samples, separate positive controls will be run in which the cells have been treated with Staurosporine and then stained with all three dyes.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work in the BSC.</p> <p>In this work, many chemicals are being used. Before starting any work, a table will be drawn in the lab book with the names of each chemical and the appropriate waste streams for each, as per the COSHH forms below. This table will be referred to at all times and will help mitigate any risks of getting waste streams mixed up.</p>	<span style="color: red; font-size: 2em;">X</span>
<p>The apoptosis wash buffer (AWB) working solution will be prepared by diluting the 15ml AWB will be diluted by adding 135ml distilled water. The 150ml working solution will then be divided into aliquots which can be frozen and used as needed.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Keep AWB container tightly shut after use. Work in the BSC.</p>	<span style="color: red; font-size: 2em;">X</span>

## Safety Method Statement (Continued)

<p>The FLICA solution will be prepared as follows:</p> <p>1) A vial of FLICA lyophilised powder will first be reconstituted with 50ul DMSO to form a stock solution that can be frozen and stored protected from light for up to 6 months. Prior to freezing, the FLICA solution (in DMSO) will be aliquoted into 10ul aliquots.</p> <p>2) When needed, the required number of aliquots of FLICA solution will be thawed and diluted using PBS (1:5 dilution) to make a working solution. Following dilution, the working solution will need to be used within 30 minutes.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work in the BSC.</p>	<p>X</p>
<p>To create positive controls, cells need to be exposed to Staurosporine solution for an appropriate number of hours specific to the cells. Initially, four samples will be set up as per the protocol below to determine how many hours of exposure is most effective to induce apoptosis. The cell samples will be exposed to staurosporine for 3, 4, 5, or 6 hours.</p> <p>Protocol for Staurosporine test:</p> <p>1) Cells will initially be harvested as per standard protocol (trypsinisation and centrifugation)</p> <p>2) Cells will then be resuspended in 20ml media and divided equally into four 15ml falcon tubes (5ml cell suspension into each tube). The number of viable cells will in each sample will be determined using a NucleoCounter with A2 slides.</p> <p>3) As per protocol, each of the samples will now be treated with 5ul of the prepared 1mM Staurosporine solution (1ul Staurosporine per ml of cell suspension).</p> <p>4) The four samples will be exposed to the Staurosporine solution for 3, 4, 5, or 6 hours respectively. To identify the optimal incubation time, after each time period, the number of viable cells in each sample will be determined using a NucleoCounter with A2 slides.</p> <p>When performing the assays, positive controls will be prepared by harvesting the cells and incubating in Staurosporine for the appropriate number of hours as determined above, prior to staining. 100,000 cells will be used for each sample.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.</p>	<p>X</p>
<p>Before the main assay is performed, it will need to be determined what concentration of the ready-made Hoechst 33342 and PI solutions, and the FLICA solution will need to be used for each 100,000 cell sample.</p> <p>This will be done by treating samples containing 100,000 cells with a series of concentrations between 10 µg/mL and 50 µg/mL of Hoechst 33342 and PI and measuring fluorescence for each sample using the NucleoCounter. For FLICA, the protocol suggests that 5ul should be used in 100ul of cell suspension. However, varying volumes of FLICA between 5ul and 20ul will be trialled to determine the optimal amount of FLICA to be used for 100,000 cell samples.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.</p>	<p>X</p>



## Safety Method Statement (Continued)

<p>The Propidium Iodide (PI) positive control will be prepared as follows:</p> <ol style="list-style-type: none"> <li>1) A healthy cell suspension containing 100,000 cells will initially be centrifuged and resuspended in 300ul 90% ethanol in PBS.</li> <li>2) Cells will be vortexed, followed by addition of 1ml apoptosis wash buffer (AWB).</li> <li>3) The dead cell suspension will then be centrifuged, supernatant aspirated, and cells resuspended in a healthy cell suspension containing 100,000 cells.</li> <li>4) From the 250 µg/mL ready-made PI solution, an appropriate amount of PI solution will then be added to the 'dead + healthy' cell suspension as per the concentration required - this was determined in the previous step. This will then be incubated in the dark for five minutes. Following incubation, fluorescence readings will be taken immediately using a flow cytometer or Nucleocounter.</li> </ol>	<p>Wear nitrile gloves, goggles, and a lab coat. Don't take whole ethanol bottle to BSC - only take a small aliquot.</p>	<p><b>X</b></p>
<p>The Propidium Iodide (PI) negative control will be prepared as follows:</p> <ol style="list-style-type: none"> <li>1) A healthy cell suspension containing 100,000 cells will initially be centrifuged and resuspended in 100ul AWB.</li> <li>2) From the 250 µg/mL ready-made PI solution, an appropriate amount of PI solution will then be added as per the concentration required - this was determined previously. The cells will be incubated in the dark for five minutes. Following incubation, fluorescence readings will be taken immediately using a flow cytometer or Nucleocounter.</li> </ol>	<p>Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>
<p>For the test samples, following the appropriate culture period, overlay media will be transferred to a falcon tube to remove any loose cells.</p>	<p>Wear nitrile gloves and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>
<p>The adherent cells will then be trypsinised, and following detachment, media will be added.</p>	<p>Wear nitrile gloves and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>
<p>The cell suspension will then be combined with the overlay media and the suspension will be centrifuged for 5mins at 200g.</p>	<p>Wear nitrile gloves and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>
<p>The supernatant will then be aspirated and cells resuspended in 2ml of media. A cell count will now be done using a NucleoCounter with A2 slides (100ul cell suspension needed for each count).</p>	<p>Wear nitrile gloves and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>
<p>The remaining cell suspension will be divided into four samples. Two of these samples will be for positive controls for which the cells will initially be treated with Staurosporine before staining with the dyes.</p> <p>The cells in the other two samples will directly be stained with FLICA and Hoechst 33342. An appropriate amount of the prepared FLICA solution and Hoechst 33342 will be used. The amounts will have been determined previously. The suspension will then be mixed by pipetting to disperse the reagents and incubated for 1hr at 37C, gently swirling the cells twice during incubation.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.</p>	<p><b>X</b></p>

## Safety Method Statement (Continued)

<p>Two wash steps will now be performed as follows:</p> <p>1) 400ul of the prepared apoptosis wash buffer will first be added to the dyed cell suspensions and mixed.</p> <p>2) The cell suspensions will then be centrifuged for 5mins at 200g and the supernatant will be aspirated.</p> <p>3) A further 400ml apoptosis wash buffer will then be added followed by centrifugation as above.</p>	<p>Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.</p>	<p>X</p>
<p>The supernatant will then be aspirated, followed by resuspension in 100 µl apoptosis wash buffer supplemented with PI (The concentration of PI to use will have been determined previously). The cells will now be analysed immediately when using the NucleoCounter. If flow cytometry is being used, the cells can be fixed using the prepared fixative. Fixed cells can be analysed up to 16hrs later. The fixative should be added at a v/v ratio of 1:5-1:10 and thhe samples should be stored at 2-8C in the dark.</p>	<p>Wear nitrile gloves, goggles, and a lab coat.</p>	<p>X</p>
<p>When using the NucleoCounter, 30 µl of each of the cell suspensions will be loaded into the chambers of the NC-Slide A2™. After placing the loaded slide on the tray of the NucleoCounter, select "Caspase Assay" and press RUN.</p>	<p>Wear nitrile gloves, goggles, and a lab coat.</p>	<p>X</p>


# COSHH Form

Reference MEME 734,735,736,737,7

Location Centre for Biological Engineering (CBE)

Originator Nishant Joglekar

Project / Activity / Task Caspase 3/7 Apoptosis Assay

<b>CHEMICAL NAME</b>						Hazard Rating <span style="border: 1px solid black; padding: 2px;">High</span>	OVERALL RISK: <span style="border: 1px solid black; padding: 5px; font-weight: bold; font-size: 1.2em;">Medium</span>
<b>Fixative</b>	CAS No. <span style="border: 1px solid black; padding: 2px;">N/A</span>	Amount used <span style="border: 1px solid black; padding: 2px;">0.1</span> <span style="border: 1px solid black; padding: 2px;">ml</span>	Period of use (hrs) <span style="border: 1px solid black; padding: 2px;">0.5</span>	The process is: <span style="border: 1px solid black; padding: 2px;">Semi Closed</span>	Physical State <span style="border: 1px solid black; padding: 2px;">Non-Volatile Liquid</span>	Exposure Potential <span style="border: 1px solid black; padding: 2px;">Low</span>	

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description	+
H302 Harmful if swallowed.	P201 Obtain special instructions before use.	X
H312 Harmful in contact with skin.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H315 Causes skin irritation.	P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.	X
H317 May cause an allergic skin reaction.	P330 Rinse mouth.	X
H319 Causes serious eye irritation.	P308 + P313 IF exposed or concerned: Get medical advice/attention.	X
H341 Suspected of causing genetic defects.	P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.	X
H350 May cause cancer.		X
H371 May cause damage to organs.		X
H335 May cause respiratory irritation.		X
H227 Combustible liquid		X
Justify the use of this chemical:	Used to fix cells following staining if flow cytometry is not being carried out within four hours of staining. This particular fixative will be used as it is provided as a ready made mixture as part of the kit. It is hence compatible with the other reagents used in the assay and does not require the addition of any diluent to adjust the concentration before use. This is beneficial as adjusting concentrations can result in errors, an increase in contamination risk, and an increase in the risk of hazards.	
How will the precautions listed above be implemented?		
Gloves, lab coat, and safety glasses will be used. Hands will thoroughly be washed after use. In case of exposure, eyes or skin will immediately be flushed with water. Fixative will be applied in a vented BSC.		
Special Storage and Containment Measures	Disposal Method	+

COSHH Form (Continued)

<p>Must be stored in a cool, dry and well-ventilated place in a tightly closed container. Once opened, container must be carefully re-sealed and kept upright to prevent leakage.</p> <p>Contact must be avoided with with strong acids and strong oxidizing substances. Incompatible with strong bases, strong acids, strong oxidizing agents, alkali metals, amines, acid chlorides, acid anhydrides, reducing agents, peroxides, isocyanates, phenol, aniline.</p>	<p>Dispose fixative containing liquid waste as hazardous chemical waste in a Winchester bottle, labelling it as a solution containing formaldehyde. Also put on the Winchester bottle, the other chemicals that the solution contains. When solution containing fixative is used in NucleoCounter slides, the slides must be disposed in cytotoxic sharps containers.</p> <p>Solid waste i.e. gloves/cloths containing traces of fixative must be disposed via the cytotoxic waste route in purple and yellow waste bags. Fixative containing pipette tips must be disposed in cytotoxic sharps containers.</p> <p>When a solution containing fixative is used in flow cytometry tubes, the polystyrene tubes must be disposed via the cytotoxic waste route in purple and yellow waste bags.</p>	<b>X</b>
<p>How will spillages be dealt with?</p>	<p><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></p>	
<p>Contain fixative and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.</p>		

<b>CHEMICAL NAME</b>						Hazard Rating	<b>X</b>	<b>OVERALL RISK:</b>
<b>FAM-DEVD-FLICA</b>						Low	<b>Low</b>	
CAS No. <input type="text" value="N/A"/>	Amount used	Period of use (hrs)	The process is:	Physical State	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential		
W.E.L. (l/ tel / stel) <input type="text"/>	1 ml	2.5	Semi Closed	Lyophilised Solid	Low			


Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	<b>X</b>
<p>How will the precautions listed above be implemented?</p> <p>N/A</p>		
Special Storage and Containment Measures	Disposal Method	+
<p>Container will be kept tightly closed in a cool, dry and well-ventilated place. Containers which are opened will be kept upright to prevent leakage.</p> <p>Will avoid contact with strong acids and strong oxidizers.</p>	<p>FLICA reagent will be reconstituted with 50ul DMSO which is cytotoxic and cannot be put down the drain. Liquid waste containing FLICA reagent must be hence disposed as non-halogenic chemical waste in a Winchester bottle.</p> <p>Contaminated solid waste i.e. gloves/cloths must be disposed via the cytotoxic waste route in purple and yellow waste bags. FLICA reagent solution containing pipette tips must be disposed in cytotoxic sharps containers.</p>	<b>X</b>
<p>How will spillages be dealt with?</p>	<p><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></p>	
<p>Contain FLICA reagent solution and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.</p>		

<b>CHEMICAL NAME</b>						Hazard Rating	<b>X</b>	<b>OVERALL RISK:</b>
<b>Apoptosis wash buffer</b>						Low	<b>Low</b>	
CAS No. <input type="text" value="N/A"/>	Amount used	Period of use (hrs)	The process is:	Physical State	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential		
W.E.L. (l/ tel / stel) <input type="text"/>	15 ml	1.5	Semi Closed	Non-Volatile Liquid	Low			

Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	<b>X</b>
<p>How will the precautions listed above be implemented?</p>		

COSHH Form (Continued)

N/A		
Special Storage and Containment Measures	Disposal Method	+
<p>Container will be kept tightly closed in a cool, dry and well-ventilated place. Containers which are opened will be kept upright to prevent leakage.</p> <p>Will avoid contact with halogenated hydrocarbons, strong acids, strong oxidizers, and metals.</p> <p>Store at 2-8C if using within one week, or freeze and use withing 6 months.</p>	<p>Any solution containing apoptosis wash buffer must be disposed as non-halogenic chemical waste in a Winchester bottle – the mixture as provided in the kit is non-hazardous due to low concentration, however, it contains sodium azide and hence must not be put down the drain.</p> <p>Solid waste i.e. gloves/cloths that is not overly contaminated can be autoclaved as the Apoptosis wash buffer as provided in the kit, is non-hazardous. If 1:20 Chemgene is used, solid waste must go down the yellow stream waste. Pipette tips with traces of Apoptosis wash buffer can be disposed in the non-cytotoxic sharps box.</p> <p>When Apoptosis wash buffer is used with other non-hazardous chemicals in NucleoCounter slides, the slides must be disposed in the non-cytotoxic sharps box.</p> <p>When Apoptosis wash buffer is used with other non-hazardous chemicals in flow cytometry tubes, the polystyrene tubes must be disposed as autoclavable waste.</p>	x
How will spillages be dealt with?	<p><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></p>	
Contain Apoptosis wash buffer and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.		

<b>CHEMICAL NAME</b>							Hazard Rating	<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;"> <span style="color: red; font-weight: bold; font-size: 1.2em;">X</span>   <b>OVERALL RISK:</b>   <div style="border: 1px solid black; padding: 2px; width: 100%; text-align: center; font-weight: bold; color: green;">Medium</div> </div>
Propidium Iodide							High	
CAS No. N/A	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		
W.E.L. (ltel / stel)	1 ml	1	Semi Closed	Non-Volatile Liquid		Low		

This chemical has a high health risk associated with it.


Hazard Statement and Description	Precaution Statement and Description	
H315 Causes skin irritation.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	x
H319 Causes serious eye irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H335 May cause respiratory irritation.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x
H341 Suspected of causing genetic defects.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	x
Justify the use of this chemical:	<p>Propidium iodide (PI) will be used as a stain to identify dead cells. As seen in the hazard statements for concentrated PI, it is a mutagen at high concentrations, however, the PI used in this assay is a ready-made mixture provided as part of the kit which is non-hazardous due to the low concentrations of PI used.</p> <p>The safety data sheet (SDS) used for concentrated PI has been provided by 'Carl Roth' - see references; whereas, the SDS for the non hazardous PI mixture that will be used for the assay is provided by 'Immunochemistry' as part of the FLICA kit.</p>	
How will the precautions listed above be implemented?		
Wear gloves, goggles, and a lab coat. Full face protection will not be required as the precautionary statements presented are for concentrated PI by 'Carl Roth', whereas, the provided PI mixture by 'Immunochemistry' that will be used is non hazardous, as seen in the SDS - see references.		
Special Storage and Containment Measures	Disposal Method	+

COSHH Form (Continued)

<p>Container will be kept tightly closed in a cool, dry and well-ventilated place. Containers which are opened will be kept upright to prevent leakage. Will avoid contact with strong acids and strong oxidizers.</p>	<p>Any solution containing Propidium Iodide must be disposed as halogenated chemical waste in a Winchester bottle - the mixture as provided in the kit is non-hazardous due to low concentration, however, it should not be put down the drain.</p> <p>Contaminated solid waste i.e. gloves/cloths can be autoclaved as Propidium Iodide, as provided, is non-hazardous. If 1:20 Chemgene is used, solid waste must go down the yellow stream waste. Pipette tips with traces of Propidium Iodide must be disposed in the non-cytotoxic sharps box.</p> <p>When Propidium Iodide is used with other non-hazardous chemicals in NucleoCounter slides, the slides must be disposed in the non-cytotoxic sharps box.</p> <p>When Propidium Iodide is used with other non-hazardous chemicals in flow cytometry tubes, the polystyrene tubes must be disposed as autoclavable waste.</p>	X
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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>
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Contain Propidium Iodide and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.

<b>CHEMICAL NAME</b>					Hazard Rating	<div style="border: 1px solid black; padding: 2px; display: inline-block;">X</div> <b>OVERALL RISK:</b> <div style="border: 1px solid black; padding: 2px; display: inline-block; width: 60px;">Medium</div>
Hoechst 33342	Amount used	Period of use (hrs)	The process is:	Physical State	High	
CAS No. N/A	0.3 ml	2.5	Semi Closed	Non-Volatile Liquid	Low	<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested
W.E.L. (l/ tel / stel)						

This chemical has a high health risk associated with it.



Hazard Statement and Description	Precaution Statement and Description	+
H315 Causes skin irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
H318 Causes serious eye damage.	P321 Specific treatment (see ... on this label).	X
H302 Harmful if swallowed.	P330 Rinse mouth.	X
H341 Suspected of causing genetic defects.	P362 Take off contaminated clothing and wash before reuse.	X
H335 May cause respiratory irritation.	P405 Store locked up.	X
	P501 Dispose of contents/container in accordance with local/regional/national/interna	X

<p>Justify the use of this chemical:</p>	<p>Hoechst 33342 will be used to stain the nuclei of cells. As seen in the hazard statements for a concentrated form of the Hoechst 33342 staining dye, it is a mutagen at high concentrations. However, the Hoechst 33342 used in this assay is a ready-made mixture provided as part of the kit which is non-hazardous due to the low concentrations of Hoechst 33342 used.</p> <p>The safety data sheet (SDS) used for the concentrated form of the Hoechst 33342 staining dye has been provided by 'Bio Rad' - see references; whereas, the SDS for the non hazardous Hoechst 33342 mixture that will be used for the assay is provided by 'Immunochemistry' - see references as part of the FLICA kit.</p>
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How will the precautions listed above be implemented?	
Wear gloves, goggles, and a lab coat. It is however, important to note that the precautionary statements presented are for the concentrated form of the Hoechst 33342 staining dye provided by 'Bio Rad', whereas, the provided Hoechst 33342 mixture by 'Immunochemistry' that will be used is non hazardous, as seen in the SDS - see references.	

COSHH Form (Continued)


Special Storage and Containment Measures	Disposal Method	
Container will be kept tightly closed in a cool, dry and well-ventilated place. Containers which are opened will be kept upright to prevent leakage. Will avoid contact with strong acids and strong oxidizers.	Any solution containing Hoechst 33342 must be disposed as halogenated chemical waste in a Winchester bottle - the mixture as provided in the kit is non-hazardous due to low concentration, however, it should not be put down the drain.  Solid waste i.e. gloves/cloths that is not overly contaminated can be autoclaved as the Hoechst 33342, as provided, is non-hazardous. If 1:20 Chemgene is used, solid waste must go down the yellow stream waste.  Pipette tips with traces of Hoechst 33342 can be disposed in the non-cytotoxic sharps box.	+
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>	+
Contain Hoechst 33342 and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.		

<b>CHEMICAL NAME</b> <b>Ethanol (Ethyl alcohol)</b>						Hazard Rating <b>High</b>	<b>OVERALL RISK:</b>  <b>Low</b>
CAS No. 64-17-5	Amount used 0.3 ml	Period of use (hrs) 0.5	The process is: Semi Closed	Physical State: Volatile Liquid	<input checked="" type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential <b>Low</b>	
W.E.L. (Itel / stel)							<b>Low</b>

Hazard Statement and Description	Precaution Statement and Description	
H225 Highly flammable liquid and vapour.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	+
H319 Causes serious eye irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	+
	P370 + P378 In case of fire: Use ... for extinction.	+
	P403 Store in a well-ventilated place.	+
	P235 Keep cool.	+

How will the precautions listed above be implemented?  
Nitrile gloves, lab coat and goggles will be worn. Hands will be washed with soap and water after use.


Special Storage and Containment Measures	Disposal Method	
Container will be kept away from all sources of ignition in a cool place. It will be kept tightly closed in a dry and well-ventilated place. When opened, container must be carefully resealed and kept upright to prevent leakage.	Dispose ethanol liquid waste via the hydrophilic organic solvent waste stream as chemical waste in Winchester bottles. Solid waste i.e. gloves/cloths containing traces of ethanol must be disposed via the cytotoxic waste route in purple and yellow waste bags. Ethanol containing pipette tips must be disposed in cytotoxic sharps containers.	+
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>	+
Use spill kit. Contain spillage, and then collect using absorbent tissue or by mopping and place in container for disposal in waste bottle or let it evaporate in fume hood. (As in current approved ethanol risk assessment ref: SAF/289)		

<b>CHEMICAL NAME</b> <b>Staurosporine solution</b>				Hazard Rating <b>Low</b>	<b>OVERALL RISK:</b>  <b>Low</b>
CAS No.	Amount used 0.2 ml	Period of use (hrs) 1	The process is: Semi Closed	Physical State: Non-Volatile Liquid	
W.E.L. (Itel / stel)					Exposure Potential <b>Low</b>



COSHH Form (Continued)

Hazard Statement and Description	Precaution Statement and Description	
No Hazard Statements applicable	No Precaution statements applicable	+
How will the precautions listed above be implemented?		
N/A		
Special Storage and Containment Measures	Disposal Method	
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature -20°C Store under inert gas away from sources of ignition	Any solution containing Staurosporine solution must be disposed as non-halogenated chemical waste in a Winchester bottle - the mixture as provided in the kit is non-hazardous due to low concentration, however, it should not be put down the drain.  Contaminated solid waste i.e. gloves/cloths can be autoclaved as Staurosporine solution, as provided, is non-hazardous.  If 1:20 Chemgene is used, solid waste must go down the yellow stream waste.  Pipette tips with traces of Staurosporine solution must be disposed in the non-cytotoxic sharps box.	+
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>	
Contain Staurosporine solution and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.		

<b>CHEMICAL NAME</b>			Hazard Rating	<b>OVERALL RISK:</b>			
<b>Dimethylsulfoxide (DMSO)</b>			High				
CAS No. 67-68-5	Amount used	Period of use (hrs)	The process is:	Physical State	<input checked="" type="checkbox"/> Eyes	Exposure Potential	<b>Low</b>
W.E.L. (Itel / stel)	2.5 ml	1	Semi Closed	Non-Volatile Liquid	<input checked="" type="checkbox"/> Skin	Low	
					<input checked="" type="checkbox"/> Inhaled		
					<input type="checkbox"/> Ingested		

Hazard Statement and Description	Precaution Statement and Description	
H315 Causes skin irritation.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	+
H319 Causes serious eye irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H335 May cause respiratory irritation.	P271 Use only outdoors or in a well-ventilated area.	x
How will the precautions listed above be implemented?		
Work will be performed in a vented BSC. Goggles, nitrile gloves and a lab coat will be worn. Hands will thoroughly be washed after use. Avoid all contact with the skin and eyes, as DMSO can be readily absorbed through the skin.		
Special Storage and Containment Measures	Disposal Method	
Must be stored in a cool, well ventilated area with the lid being tightly closed.	Any solution containing DMSO must be disposed as non-halogenated chemical waste in a Winchester bottle. Contaminated solid waste i.e. gloves/cloths must be disposed via the cytotoxic waste route in purple and yellow waste bags. Pipette tips containing traces of DMSO must be disposed in cytotoxic sharps containers. When DMSO is in solution used in NucleoCounter slides, the slides must be disposed in cytotoxic sharps containers. When a solution containing DMSO is used in flow cytometry tubes, the polystyrene tubes must be disposed via the cytotoxic waste route in purple and yellow waste bags.	+



## COSHH Form (Continued)

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>
Contain DMSO and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene. With any spillage only likely to be a few drops, virkon will not be required.	

+ Add another chemical

Statement of work (Process to be undertaken)

Caspase 3/7 apoptosis assay using NucleoCounter of flow cytometer

Show image

Personal protection requirements not covered in the precaution statements above.

Show covers

Sources of information and references

SOP039; SOP038; <https://immunochemistry.com/wp-content/uploads/2016/05/93-94-FAMDEVD-KIT-SDS-2.pdf>; <https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=S6942&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fs6942%3Flang%3Den>; [https://store.apolloscientific.co.uk/storage/msds/BID1200\\_msds.pdf](https://store.apolloscientific.co.uk/storage/msds/BID1200_msds.pdf); <https://immunochemistry.com/wp-content/uploads/2016/05/F18-91-6-D-1.pdf>; <https://marketing.chemometec.com/acton/attachment/21287/f-00f0/1/-/-/-/994-3021-FLICA-Caspase-Assay.pdf>; [https://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB\\_ENG\\_1351304.pdf](https://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB_ENG_1351304.pdf); <https://www.carlroth.com/medias/SDB-CN74-MT-EN.pdf?context=bWFzdGVyfHNIY3VyaXR5RGF0YXNoZWV0c3wyMDAwNjZ8YXBwbGljYXRpb24vcGRmfHNIY3VyaXR5RGF0YXNoZWV0cy9oOTYvaDI0Lzgz5Njk2MDEyODYxNzQucGRmfDMYNTQ3OGU4M2M0MzcxMzUzNjYwZGU2OTZkMWM4NmIxYWZiMjJjODRmNDY1MTM0MzJmYTkwNTA1NTg4ZDIwYTk>; <https://immunochemistry.com/wp-content/uploads/2016/06/Staurosporine-Product-Insert.pdf>

Reference to **existing approved** Risk Assessment

SAF/289

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 6530

Method Statement

SAF/MEME 6530

COSHH Assessment

MEME 734,735,736,737,

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:

19/08/2021

Review comments