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	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
Project / Activity / Task	Annexin V Assay
Supervisor Name	Karen Coopman and Elizabeth Ratcliffe

Version : 2.19

Loughborough University
Department of Chemical Engineering

Risk Assessment

Reference: SAF/MEME6531

Location: Centre for Biological Engineering

Originator: Nishant Joglekar

Project / Activity / Task: Annexin V Assay

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

Category 1: Machinery & work equipment:				
Design and Construction	Mechanical hazards	Electrical hazards	Radiation hazards	+
N/A	N/A	Electrical test lables current	N/A	x
Category 2: Workplace				+
N/A				x
Category 3: Hazardous and/or Harmful substances				+
Flammable substances - ethanol is flammable; refer to COSHH below				x
Irritant substances - DMSO and ethanol are irritants; refer to COSHH forms below				x
Category 4: Work activity				+
N/A				x
Category 5: Work organisation				+
N/A				x

Explain the risks associated with these hazards

People / Groups at risk: Operator only — x

Enter risk details here:-	Impact	Probability	Risk Score
Exposure to harmful substances	Harmful	Unlikely	Medium

What are the control measures?	Lowers Impact	Lowers Probability	+
Appropriate PPE will be worn	Significantly	Significantly	x
Spillages will be dealt with immediately as per risk assessment	Significantly	Significantly	x
Work will be done in a BSC	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:-	Impact	Probability	Risk Score
Risk of fire due to ethanol	Very Harmful	Highly Unlikely	Medium

Process Risk Assessment Form (Continued)

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: Everyone in the room — x

Enter risk details here:-	Impact	Probability	Risk Score
Exposure to hazardous substances for others in the labs	Harmful	Highly Unlikely	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: Operator and people in proximity — x

Enter risk details here:-	Impact	Probability	Risk Score
Electrical hazards - visual inspection required	Harmful	Highly Unlikely	Low

What are the control measures?	Lowers Impact	Lowers Probability	+
All electrical equipment in the area is PAT tested annually to ensure electrical safety, and a quick 'visual inspection' is carried out before any work begins. This ensures that any damage to equipment casing or wires which could lead to them being unsafe is checked before use. Upon discovering damage, technicians take the equipment out of use using a 'lock out tag out' system.	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	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
Total	0	1	0	3	0	0	4

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

Loughborough University

Reference: SAF/MEME6531

Location: Centre for Biological Engineering

Originator: Nishant Joglekar

Project / Activity / Task: Annexin V Assay

What equipment will be used in this activity?	+
Heating block	x
Nucleocounter	x
Flow cytometer	x
BSC	x
Centrifuge	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)	+
Annexin V-CF488A conjugate (part of kit)	x
Annexin V binding buffer (part of kit)	x
Propidium Iodide (part of kit)	x
Hoechst 33342 (ready-made solution part of FLICA assay kit - this can also be used for this assay - compatible)	x
Staurosporine solution	x
DMSO	x
Ethanol	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)

Make sure chemical containers are tightly closed, upright, and kept in a well-ventilated area.	x
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
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
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	x
https://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB_ENG_1351304.pdf	x
https://biotium.com/wp-content/uploads/2016/12/PI-30061.pdf	x
https://biotium.com/wp-content/uploads/2017/10/SDS-30061.pdf	x
https://marketing.chemometec.com/acton/attachment/21287/f-00ef/1/-/-/-/-/994-3017-Annexin-V-Assay.pdf	x
SAF/289 - Reference of existing approved ethanol risk assessment	x
https://www.carlroth.com/medias/SDB-CN74-MT-EN.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wyMDAwNjZ8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oOTYvaDI0Lzg5Njk2MDEyODYxNzQucGRmfDMyNTQ3OGU4M2M0MzcxMzUzNjYwZGU2OTZkMWM4NmIxYWZiMjJjODRmNDY1MTM0MzJmYTkwNTA1NTg4ZDIwYTk	x
https://store.apolloscientific.co.uk/storage/msds/BID1200_msds.pdf	x
https://immunochemistry.com/wp-content/uploads/2016/05/93-94-FAMDEVD-KIT-SDS-2.pdf	x

Detailed sequential description of the process

Process step	Precautionary measures and comments	+

Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
The following samples will be prepared: 1) A positive Annexin V-CF488A control in which apoptosis has been induced in the cells using staurosporine prior to staining with just Annexin V-CF488A. 2) A negative Annexin V-CF488A control in which healthy cells are stained with just Annexin V-CF488A. 3) A positive Hoechst 33342 control in which apoptosis has been induced in the cells using staurosporine prior to staining with just Hoechst 33342. 4) A negative Hoechst 33342 control in which healthy cells are stained with just Hoechst 33342. 5) A positive Annexin V-CF488A and Hoechst 33342 control in which apoptosis has been induced in the cells using staurosporine prior to staining with both Annexin V-CF488A and Hoechst 33342. 6) A negative Annexin V-CF488A and Hoechst 33342 control in which healthy cells are stained with both Annexin V-CF488A and Hoechst 33342. 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. 8) An unlabelled negative control in which cells are healthy and no staining has been performed where DMSO has been used as a vehicle. 9) A positive PI control in which the cells have been killed using ethanol prior to staining with just PI. 10) A negative PI control in which healthy cells are stained with just PI. 11) Test sample which will involve staining with Annexin V-CF488A, Hoechst 33342 and PI. 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. 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.	Wear nitrile gloves, goggles, and a lab coat. Work in the BSC. 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.	x
The provided 5X Annexin V Binding Buffer will be diluted using distilled water at a ratio 1:5 to prepare approximately 1 mL of 1X Annexin V Binding Buffer for each sample to be stained.	Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.	x
A working solution of PI will be prepared by diluting the PI at a ratio of 1:10 using the prepared 1X Binding Buffer.	Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.	x

Safety Method Statement (Continued)

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. Protocol for Staurosporine test: 1) Cells will initially be harvested as per standard protocol (trypsinisation and centrifugation) 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. 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). 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. 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.	Wear nitrile gloves, goggles and a lab coat.	x
Before the main assay is performed, it will need to be determined what concentration of Hoechst 33342, PI, and Annexin V-CF488A will need to be used for each 100,000 cell sample. 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 Annexin V-CF488A, the protocol suggests that 2ul of Annexin V-CF488A should be added to cells in 100ul binding buffer. However, varying volumes of Annexin between 2ul and 10ul will be trialled to determine the optimal amount of Annexin V-CF488A to be used for 100,000 cell samples.	Wear nitrile gloves, goggles, and a lab coat.	x

Safety Method Statement (Continued)

The Propidium Iodide (PI) positive control will be prepared as follows: 1) A healthy cell suspension containing 100,000 cells will initially be centrifuged and resuspended in 300ul 90% ethanol in PBS. 2) Cells will be vortexed, followed by addition of 1ml apoptosis binding buffer. 3) The dead cell suspension will then be centrifuged, supernatant aspirated, and cells resuspended in a healthy cell suspension containing 100,000 cells. 4) 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.	Wear nitrile gloves, goggles, and a lab coat. Don't take whole ethanol bottle to BSC - only take a small aliquot.	x
The Propidium Iodide (PI) negative control will be prepared as follows: 1) A healthy cell suspension containing 100,000 cells will initially be centrifuged and resuspended in 100ul binding buffer. 2) 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.	Wear nitrile gloves, goggles, and a lab coat.	x
For the test samples, following the appropriate culture period, overlay media will be transferred to a falcon tube to remove any lose cells.	Wear nitrile gloves and a lab coat. Work inside a BSC.	x
The adherent cells will then be trypsinised, and following detachment, media will be added.	Wear nitrile gloves and a lab coat. Work inside a BSC.	x
The cell suspension will then be combined with the overlay media and the suspension will be centrifuged for 5mins at 200g.	Wear nitrile gloves and a lab coat.	x
The supernatant will then be aspirated and cells resuspended in 2ml of binding buffer. A cell count will now be done using a NucleoCounter with A2 slides (100ul cell suspension needed for each count).	Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC for aspiration and resuspension.	x
The remaining cell suspension (cells in binding buffer) will then 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. The cells in the other two samples will directly be stained with an appropriate amount of of Annexin V-CF488A and Hoechst 33342. The amounts will have been determined previously. Following mixing, the cells will be incubated at 37C on a heating block for 15mins.	Wear nitrile gloves, goggles, and a lab coat. Be careful not to touch the heating block when hot.	x
Following incubation, the stained cells will be spun down at 400g for 5 minutes and the supernatant removed using a pipette.	Wear nitrile gloves and a lab coat. Work inside a BSC.	x
The cells will now be washed by resuspending in 300ul of Annexin V binding buffer and spinning down again at 400g for 5 minutes, followed by the removal of the supernatant using a pipette.	Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.	x
The previous step will be repeated.	Wear nitrile gloves, goggles, and a lab coat. Work inside a BSC.	x

Safety Method Statement (Continued)

When measuring flourescence using the NucleoCounter, the cells will now be resuspended in 100ul Annexin V binding buffer supplemented with PI (The concentration of PI to use will have been determined previously). Following resuspension, the cells will be analysed immediately. 'Annexin V Assay' will be selected on the NucleoCounter.	Wear nitrile gloves, goggles, and a lab coat.	x
When measuring flourescence using flow cytometry, each of the four samples (two of which are positive controls) will be transferred to flow cytometry tubes.	Wear nitrile gloves, goggles, and a lab coat.	x
Appropriate amounts of CF488A-Annexin V and PI working solution as determined previously will then be added and the cells will be incubated for 15-30 minutes in the dark - perform the incubation on ice. Hoechst 33342 is not required for flow cytometry. 400 uL of binding buffer will then be added to each tube and flow cytometry will be performed within 30 minutes of staining.	Wear nitrile gloves, goggles, and a lab coat.	x

Loughborough University
Department of Chemical Engineering

COSHH Form

Reference: MEME741,742,743,744,73

Location: Centre for Biological Engineering

Originator: Nishant Joglekar

Project / Activity / Task: Annexin V Assay

CHEMICAL NAME

Annexin V-CF488A conjugate

CAS No. N/A

W.E.L. (Itel / stel)

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential	Hazard Rating
0.04	ml	1	Semi Closed	Non-Volatile Liquid	☐ Eyes ☐ Skin ☐ Inhaled ☐ Ingested	Low	Low

OVERALL RISK: Low

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
Keep container tightly closed in a dry and well-ventilated place. Store at 4C.	Any solution containing Annexin V-CF488A conjugate must be disposed as halogenated waste in a Winchester bottle – the mixture as provided in the kit is non-hazardous due to low concentrations, however, it should not be put down the drain as it contains sodium chloride. Contaminated solid waste i.e. gloves/cloths can be autoclaved as Annexin V-CF488A conjugate, 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 Annexin V-CF488A conjugate must be disposed in the non-cytotoxic sharps box. When Annexin V-CF488A conjugate is used with other non-hazardous chemicals in NucleoCounter slides, the slides must be disposed in the non-cytotoxic sharps box. When Annexin V-CF488A conjugate is used with other non-hazardous chemicals in flow cytometry tubes, the polystyrene tubes must be disposed as autoclavable waste.

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

Contain Annexin V-CF488A conjugate and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.

CHEMICAL NAME

Annexin V binding buffer

CAS No. N/A

W.E.L. (Itel / stel)

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential	Hazard Rating
0.9	ml	1	Semi Closed	Non-Volatile Liquid	☐ Eyes ☐ Skin ☐ Inhaled ☐ Ingested	Low	Low

OVERALL RISK: 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?	
N/A	

Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place. Store at 4C.	Any solution containing Annexin V buffer must be disposed as halogenated waste in a Winchester bottle – the mixture as provided in the kit is non-hazardous due to low concentrations, however, it should not be put down the drain. Annexin V binding buffer can be disposed down the biological waste route. Contaminated solid waste i.e. gloves/cloths can be autoclaved as Annexin V binding buffer is non-hazardous. If 1:20 Chemgene is used, solid waste must go down the yellow stream waste. Pipette tips with traces of Annexin V binding buffer must be disposed in the non-cytotoxic sharps box. When Annexin V binding buffer is used with other non-hazardous chemicals in NucleoCounter slides, the slides must be disposed in the non-cytotoxic sharps box. When Annexin V binding buffer is used with other non-hazardous chemicals in flow cytometry tubes, the polystyrene tubes must be disposed as autoclavable 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*	
Contain Annexin V binding buffer and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.		

CHEMICAL NAME

Propidium Iodide

CAS No. N/A

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium X

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.02	ml	1	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	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. Remov	x
Justify the use of this chemical:	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. 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 Annexin V kit.	

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
Keep container tightly closed in a dry and well-ventilated place. Store at 4C.	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. 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. When Propidium Iodide is used with other non-hazardous chemicals in NucleoCounter slides, the slides must be disposed in the non-cytotoxic sharps box. When Propidium Iodide is used with other non-hazardous chemicals in flow cytometry tubes, the polystyrene tubes must be disposed as autoclavable waste.
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*

Contain Propidium Iodide and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.

CHEMICAL NAME

Hoechst 33342

CAS No. 23491-52-3

W.E.L. (Itel / stel)

Hazard Rating: High

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.3	ml	1	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☑ Ingested	Low

OVERALL RISK: Medium

This chemical has a high health risk associated with it.

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

Justify the use of this chemical:	Used as a DNA-binding dye as per protocol in order to stain the total cell population when performing the Annexin V apoptosis assay on the NucleoCounter. 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 FLICA kit which is non-hazardous due to the low concentrations of Hoechst 33342 used. 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.

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.

Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place. Store at 4C.	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	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*
Contain Hoechst 33342 and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.	

CHEMICAL NAME

Ethanol (Ethyl alcohol)

CAS No. 64-17-5

W.E.L. (Itel / stel)

Hazard Rating: High

X

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.3	ml	0.5	Semi Closed	Volatile Liquid	☑ Eyes ☐ Skin ☐ Inhaled ☐ Ingested	Low

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.	x
H319 Causes serious eye irritation.	P403 Store in a well-ventilated place.	x
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	x
	P370 + P378 In case of fire: Use … for extinction.	x
	P235 Keep cool.	x

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.

COSHH Form (Continued)

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?	*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*
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)	

CHEMICAL NAME

Staurosporine solution

CAS No.

W.E.L. (Itel / stel)

Amount used		Period of use (hrs)	The process is:	Physical State		Hazard Rating	Exposure Potential
0.2	ml	1	Semi Closed	Non-Volatile Liquid	☐ Eyes ☐ Skin ☐ Inhaled ☐ Ingested	Low	Low

OVERALL RISK: Low

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 igntion	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?	*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*
Contain Staurosporine solution and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.	

CHEMICAL NAME

Dimethylsulfoxide (DMSO)

CAS No. 67-68-5

W.E.L. (Itel / stel)

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

OVERALL RISK: Low

Hazard Statement and Description	Precaution Statement and Description
H315 Causes skin irritation.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

COSHH Form (Continued)

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.
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*
Contain DMSO and wipe the spill area using an inert absorbent cloth. Additionally, clean with 1:20 Chemgene.	

+ Add another chemical

Statement of work (Process to be undertaken)

Annexin V apoptosis assay

Show Image

Personal protection requirements not covered in the precaution statements above.

Shoe covers

Sources of information and references

SOP039; SOP038; 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://www.bio-rad.com/webroot/web/pdf/WWMSDS/LSGC/GB/GB_ENG_1351304.pdf; https://store.apolloscientific.co.uk/storage/msds/BID1200_msds.pdf; https://biotium.com/wp-content/uploads/2016/12/PI-30061.pdf; https://biotium.com/wp-content/uploads/2017/10/SDS-30061.pdf; https://marketing.chemometec.com/acton/attachment/21287/f-00ef/1/-/-/-/-/994-3017-Annexin-V-Assay.pdf; https://www.carlroth.com/medias/SDB-CN74-MT-EN.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wyMDAwNjZ8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oOTYvaDI0Lzg5Njk2MDEyODYxNzQucGRmfDMyNTQ3OGU4M2M0MzcxMzUzNjYwZGU2OTZkMWM4NmIxYWZiMjJjODRmNDY1MTM0MzJmYTkwNTA1NTg4ZDIwYTk; https://immunochemistry.com/wp-content/uploads/2016/05/93-94-FAMDEVD-KIT-SDS-2.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: Karen Coopman — Digitally signed by Karen Coopman Date: 2020.08.19 10:39:29 +01'00'

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME6531	SAF/MEME6531	MEME741,742,743,744,7

DSO Signature: Digitally signed by David Green Date: 2020.08.25 09:08:11 +01'00' Adobe Acrobat Reader version: 2020.009.20074

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

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

Next Review: 19/08/2021

Review comments