

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	CBE; H23, H30, H34
Project / Activity / Task	Detecting Treg cells following co-culture of CD4+ T cells and MSCs/ culture of T cells in MSC conditioned media
Supervisor Name	Dr Karen Coopman

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	Crushing	Direct contact	Lasers	+
		Electrical test cables current		X
Category 2: Workplace				
Risk of asphyxiation (Oxygen depletion)				X
Slips/Trips/Falls on the level				X
Category 3: Hazardous and/or Harmful substances				
Hazardous substances - refer to SAF/MEME/6698				X
Corrosive substances - refer to SAF/MEME/6698				X
Biological substances (Infection) - cell work				X
Cancer causing substances - refer to COSHH below for Fixation/Permeabilization Solution 1				X
Toxic substances - refer to COSHH below for Fixation/Permeabilization Solution 1				X
Exposure to Covid-19				X
Category 4: Work activity				
Highly repetitive actions - standard cell culture can be repetitive				X
Lone working out of hours				X
Category 5: Work organisation				
N/A				X

Explain the risks associated with these hazards				
People / Groups at risk	<input type="text" value="Operator only"/>			X
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Laser radiation"/>	<input type="text" value="Very Harmful"/>	<input type="text" value="Highly Unlikely"/>	Medium	
What are the control measures?	<input type="text" value="Lowers Impact"/>	<input type="text" value="Lowers Probability"/>	+	

Process Risk Assessment Form (Continued)

Refer to SAF/MEME/6698 (Lasers are used in the flow cytometer; there are light shields built in to the cytometer to prevent exposure, direct eye exposure to the laser will be prevented/avoided)	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Electrical shock - refer to SAF/MEME/6698	Very Harmful	Highly Unlikely	Medium	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Refer to SAF/MEME/6698 (Instrument will be turned off before attempting to remove the flow cell Visual inspection of electrical cables and connectors before use Ensure where PAT testing is required the equipment is within current inspection date)	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Crushing - refer to SAF/MEME/6698	Harmful	Highly Unlikely	Low	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Refer to SAF/MEME/6698 (When opening the sample tray, the 'Eject Tray' button in the guava Software will be used - fingers will never be used to open the tray; the area in front of the tray will be kept clear when opening)	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Biological hazard - MSCs and T cells used	Very Harmful	Highly Unlikely	Medium	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Refer to BRA (All waste will be disposed appropriately as per SOP003)	Significantly	Significantly	x	
			Residual Risk	
			Low	
People / Groups at risk	Operator only			x
Enter risk details here:-	Impact	Probability	Risk Score	
Toxic cancer/genetic defects causing substance used	Very Harmful	Highly Unlikely	Medium	
What are the control measures?	Lowers Impact	Lowers Probability	+	
Refer to COSHH below for Fixation/Permeabilization Solution 1	Significantly	Significantly	x	

Process Risk Assessment Form (Continued)

			Residual Risk
			Low
People / Groups at risk	Everyone in the room		X
Enter risk details here:-	Impact	Probability	Risk Score
Risk of asphyxiation - Use of liquid nitrogen vapour	Very Harmful	Highly Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
There is an oxygen monitor present which is checked regularly and will alarm when the oxygen level falls.	Significantly	Significantly	X
Door will be propped open when using the cryobanks for thawing cells. Must be trained in use/ handling of cryogenic materials	Significantly	Significantly	X
			Residual Risk
			Low
People / Groups at risk	Operator and people in proximity		X
Enter risk details here:-	Impact	Probability	Risk Score
Slips trips and falls	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Working areas to be kept clean and tidy - any floor based obstacles or hazards should be cleared away	Slightly	Moderately	X
			Residual Risk
			Low
People / Groups at risk	Operator only		X
Enter risk details here:-	Impact	Probability	Risk Score
Lone working	Slightly Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Should out of hours working be required, permission to work out of hours must be obtained prior to work commencing. Sign in using the lone working Power App (https://www.lboro.ac.uk/services/health-safety/loneworking/). It is advisable to also inform security so that they are aware of your location on campus for the duration of your lone working/out of hours . Inform academic supervisor and a colleague of intention to lone work and state duration of stay. Lone working duty officer will be appointed. Ensure you have mobile phone on person at all times. Always remember to log out of lone working app when leaving building at completion of the work	None	Moderately	X
			Residual Risk
			Low
People / Groups at risk	Everyone in the room		X
Enter risk details here:-	Impact	Probability	Risk Score
Exposure to Covid-19	Very Harmful	Highly Unlikely	Medium

Process Risk Assessment Form (Continued)

What are the control measures?	Lowers Impact	Lowers Probability	+
Follow all national, local and University Covid-19 guidelines, and respect CBE Lab rules. Frequent washing / sanitizing of hands / gloves to be carried out. Touch points and surfaces to be cleaned / wiped down after use. Distancing should be 2 metre, but 1M+ is allowed where all concerned are wearing face coverings Check local Covid tier rating	None	Moderately	x
			Residual Risk
			Low
+ Add another Risk			

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

Personnel Group	Maximum (Task setup/ Re-configuration)	High (Performing the task)	Medium (Observing the task)	Low (Present, but not involved)	Lone Working (Out of hours)	No Exposure Permitted	Total
Academic Staff	0	0	0	0	0	0	0
Technical Staff	1	1	0	1	0	0	3
Research Staff (PDRA)	1	1	0	1	0	0	3
Research Students (PhD)	1	1	0	1	0	0	3
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	3	3	0	3	0	0	9

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/6749

Location CBE; H23, H30, H34 Originator Nishant Joglekar

Project / Activity / Task Detecting Treg cells following co-culture of CD4+ T cells and MSCs/culture of T cells in MSC conditioned media

What equipment will be used in this activity?	+
Biological safety cabinet (BSC)	X
Water bath	X
Centrifuge	X
Flow cytometer	X

What training must be completed to do this activity?	+
Aseptic technique	X
Standard cell culture training	X
Standard CBE training	X
Flow cytometry training	X

What chemicals are being used? (These must be included in the COSHH Form)	+
Guava Instrument Cleaning Fluid (ICF) - refer to SAF/MEME/669 for COSHH	X
Sodium hypochlorite solution (bleach) - refer to SAF/MEME/669 for COSHH	X
ImmunoCult™ HumanCD3/CD28 T Cell Activator - non-hazardous	X
Recombinant human IL-2 - non-hazardous	X
Fixation/Permeabilization Solution 1 (part of kit) - see COSHH below	X
Fixation/Permeabilization Solution 2 (part of kit) - non-hazardous	X
10x Permeabilization Buffer (part of kit) - non-hazardous	X

Spill and accident procedures.	+
For ICF/sodium hypochlorite solution spillages, refer to SAF/MEME/669	X
For small spillages of any non-hazardous substances, use an absorbent cloth / tissue with 1:20 chemgene to clear up the spillage and dispose of the cloth / tissue in the yellow stream waste.	X

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
Make sure all chemical containers are tightly closed and upright. Leave flow cytometer/BSC on, and exit the laboratory. Remove all contaminated PPE and wash hands 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.	+
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Safety Method Statement (Continued)

SAF/MEME/669	X
BRA	X
https://www.miltenyibiotec.com/GB-en/products/treg-detection-kit-human.html#gref - for treg detection kit data sheet	X
https://www.stemcell.com/product-portfolios/t-cell-research/cell-activation-and-expansion/immunocult-human-cd3-cd28-t-cell-activator.html#section-product-documents - for ImmunoCult™ Human CD3/CD28 T Cell Activator SDS and information sheet	X
SDS for Formaldehyde - see attached to email	X
SDS for Treg Detection Kit, human - see attached to email	X
https://www.stemcell.com/products/human-recombinant-il-2.html#section-product-documents - for Recombinant human IL-2 SDS and information sheet	X
https://www.fishersci.co.uk/store/msds?partNumber=10010240&productDescription=5LT+Methanol%2C+Certified+AR+for+analysis&countryCode=GB&language=en	X
SOP039, SOP003, SOP138	X

Detailed sequential description of the process

Process step	Precautionary measures and comments	
<p>For co-culture:</p> <p>1) Either thaw and culture MSCs from working cell bank as per standard protocol or use cells that are already in culture and seed in 24 well plates and culture to 80-90% confluency.</p> <p>2) When MSCs reach confluency, replace media with a cell suspension containing freshly thawed CD4+ T cells in media supplemented with human rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator (1x10⁶ T cells/well) (1ml media per well). T cells thawed as per standard protocol.</p> <p>3) At each 24hr interval, count the number of T cells to monitor proliferation and using the TREG detection kit and the Guava flow cytometer to detect CD45+/CD4+/CD25+/CD127^{dim}/neg/FoxP3+ cells, determine the change in the number of Tregs. Follow the manufacturer's instructions for the detection of Tregs using the kit and the flow cytometer. Perform the tests every 24hrs for six days.</p> <p>4) If T cell density gets above 2.5x10⁶ cells/ml, add extra media to adjust seeding density back to 1x10⁶cells.</p> <p>5) Perform co-cultures in triplicates and also include controls. Controls to include:</p> <ul style="list-style-type: none"> - MSCs without T cells - MSCs with T cells without rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator - T cells without MSCs - Blank T cell media 	<p>Wear gloves, lab coat and shoe covers at all times.</p> <p>When performing flow cytometry, also wear safety glasses and keep chemical containers tightly closed. Observe and respect CBE local lab rules/SOPs</p>	+
<p>For preparing MSC conditioned media (MSC CM):</p> <p>1) Thaw and culture MSCs from working cell bank as per standard protocol in 24 well plates to 80-90% confluency.</p> <p>2) Replace medium with serum free media for 72hrs.</p> <p>3) Collect medium using ultracentrifugation as per standard protocol and store at -80C to use as required.</p>	<p>Wear gloves, lab coat and shoe covers at all times.</p>	X

Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
<p>Culturing T cells in MSC CM:</p> <p>1) Thaw MSC CM and culture CD4+ T cells in MSC CM (1x10⁶ T cells/well) (1ml media per well).</p> <p>2) When culturing, add human rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator to the MSC CM.</p> <p>3) As with co-culture, count cells each day and to monitor proliferation and using the TREG detection kit and the Guava flow cytometer to detect CD45+/CD4+/CD25+/CD127^{dim}/neg/FoxP3+ cells, determine the change in the number of Tregs.</p> <p>4) After three days, perform a media change as per the standard protocol.</p> <p>5) If T cell density gets above 2.5x10⁶ cells/ml, add extra MSC CM to adjust seeding density back to 1x10⁶cells.</p> <p>6) Perform samples in triplicates and also include controls. Controls to include:</p> <ul style="list-style-type: none"> - T cells in standard RPMI media with rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator - T cells in standard RPMI media without rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator - T cells in MSC CM without rIL-2 and ImmunoCult™ Human CD3/CD28 T Cell Activator - Blank MSC CM without T cells 	<p>Wear gloves, lab coat and shoe covers at all times.</p> <p>When performing flow cytometry, also wear safety glasses and keep chemical containers tightly closed.</p>	<p style="text-align: center;">+</p> <p style="text-align: center;">X</p>
<p>Performing flow cytometry:</p> <p>All flow cytometry work to detect treg cells will be performed using the treg identification kit and the Guava flow cytometer as per the manufacturer's instructions (see references).</p> <p>Briefly, initially working solutions of the Fixation/Permeabilization Solutions and the Permeabilization Buffer will be prepared. The cells will then be surface stained for CD45, CD4, CD25, and CD127 by adding CD45-VioBlue®, CD4-VioGreen™, CD25-VioBright™ 515, and CD127-PE antibodies, after which intracellular staining will be performed with the Anti-FoxP3-Vio667 antibody to detect for FoxP3. Flow cytometry will then be used to detect CD45+/CD4+/CD25+/CD127^{dim}/neg/FoxP3+ cells - all staining procedures will be done in the dark in a fridge.</p>	<p>Wear gloves, lab coat and shoe covers at all times.</p> <p>When performing flow cytometry, also wear safety glasses and keep chemical containers tightly closed.</p>	<p style="text-align: center;">+</p> <p style="text-align: center;">X</p>

COSHH Form

Reference SAF/MEME/982 - 989 & 9

Location CBE; H23, H30, H34

Originator Nishant Joglekar

Project / Activity / Task Detecting Treg cells following co-culture of CD4+ T cells and MSCs/culture of T cells in MSC conditioned media

CHEMICAL NAME				Hazard Rating		OVERALL RISK: Low
Recombinant human IL-2				Low		
CAS No.		Amount used	Period of use (hrs)	The process is:	Physical State	Exposure Potential Low
W.E.L. (Itel / stel)		0.00005 g	0	Semi Closed	Lyophilised Solid	
				<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		

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 and store at -20C (store both lyophilised solid, and reconstituted solution at -20C)	Eppendorf tubes with traces of reagent should be put down yellow stream waste (reagent should not enter drain)	+
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. Click here to see spill procedures</i>	+
Spray 1:20 chemgene on spill area and wipe with tissue		

CHEMICAL NAME				Hazard Rating		OVERALL RISK: Low
ImmunoCult™ HumanCD3/CD28 T Cell Activator				Low		
CAS No.		Amount used	Period of use (hrs)	The process is:	Physical State	Exposure Potential Low
W.E.L. (Itel / stel)		2 ml	0	Semi Closed	Non-Volatile Liquid	
				<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		

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, cool and well-ventilated place	Any tissues with traces of Chemgene used to clean spillages should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of ImmunoCult™ HumanCD3/CD28 T Cell Activator should be disposed in orange non-cytotoxic sharps containers. Other solid waste and solutions containing ImmunoCult™ HumanCD3/CD28 T Cell Activator along with other non-hazardous substances can be disposed down the biological waste route.	+
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. Click here to see spill procedures</i>	+

COSHH Form (Continued)

Any spillages will be less than 2ml and within a BSC. For a small spill such as this, the spillage can be cleaned up using a tissue. 1:20 Chemgene will then be sprayed over the spill area which will also then be wiped down using a tissue. The tissues must then be disposed as cytotoxic solid waste.

CHEMICAL NAME Fixation/Permeabilization Solution 1				Hazard Rating High	X
CAS No. <input type="text"/>	Amount used <input type="text" value="1"/> <input type="text" value="ml"/>	Period of use (hrs) <input type="text" value="3"/>	The process is: <input type="text" value="Semi Closed"/>	Physical State <input type="text" value="Non-Volatile Liquid"/>	OVERALL RISK: Medium
W.E.L. (Itel / stel) <input type="text"/>				<input type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested Exposure Potential Low	

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description	+
H302 Harmful if swallowed.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	X
H317 May cause an allergic skin reaction.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H341 Suspected of causing genetic defects.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
H350 May cause cancer.	P308 + P313 IF exposed or concerned: Get medical advice/attention.	X
Justify the use of this chemical:	This is a reagent that is provided as part of the kit required to detect Treg cells.	

How will the precautions listed above be implemented?
 Gloves, safety glasses and lab coat will be worn.
 In case of skin contact, water and soap will be used, and in case of any skin irritation, medical treatment will be sought and contaminated clothing removed.
 In case of eye contact, Eyes will be rinsed immediately carefully and thoroughly with eye-bath or water. In case of persistent symptoms, ophthalmologist will be consulted.

Special Storage and Containment Measures	Disposal Method	+
Container must be kept tightly shut in a fridge (2-8C). Container should not be kept in a freezer or exposed to sunlight.	Any tissues/stripped with traces of Fixation/Permeabilization Solution 1 should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of Fixation/Permeabilization Solution 1 should be disposed in purple cytotoxic sharps containers.	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. Click here to see spill procedures</i>	




Any spillages are likely to be less than 1-2ml and within a BSC. For a small spill such as this, the spillage can be cleaned up using a tissue. 1:20 Chemgene will then be sprayed over the spill area which will also then be wiped down using a tissue. The tissues must then be disposed as cytotoxic solid waste.

CHEMICAL NAME Formaldehyde 1 - < 5% (part of Fixation/Permeabilization)				Hazard Rating High	X
CAS No. <input type="text" value="50-00-0"/>	Amount used <input type="text" value="1"/> <input type="text" value="ml"/>	Period of use (hrs) <input type="text" value="3"/>	The process is: <input type="text" value="Semi Closed"/>	Physical State <input type="text" value="Non-Volatile Liquid"/>	OVERALL RISK: Medium
W.E.L. (Itel / stel) <input type="text"/>				<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested Exposure Potential Low	

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description	+
H341 Suspected of causing genetic defects.	P201 Obtain special instructions before use.	X
H350 May cause cancer.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled	P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.	X

COSHH Form (Continued)

H314 Causes severe skin burns and eye damage.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
H317 May cause an allergic skin reaction.	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	X
H370 Causes damage to organs.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	X
	P307 + P311 IF exposed: Call a POISON CENTER or doctor/physician.	X
Justify the use of this chemical:	Formaldehyde is a component of the Fixation/Permeabilization Solution 1 provided in the kit - it is not provided as a chemical in a separate bottle/vial	
How will the precautions listed above be implemented?		
Refer to 'Precautions' section above for 'Fixation/Permeabilization Solution 1' - formaldehyde is just a component of the solution		
Special Storage and Containment Measures	Disposal Method	+
Refer to storage considerations above for 'Fixation/Permeabilization Solution 1' - formaldehyde is just a component of the solution	Refer to 'disposal section' above for 'Fixation/Permeabilization Solution 1' - formaldehyde is just a component of the solution	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.</i> Click here to see spill procedures	
Refer to 'spillages' section above for 'Fixation/Permeabilization Solution 1' - formaldehyde is just a component of the solution		
CHEMICAL NAME	  	<div style="border: 1px solid black; padding: 2px; display: inline-block;">Hazard Rating</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; color: red; font-weight: bold;">High</div>
Methanol 1 - < 5% (part of Fixation/Permeabilization)	<div style="display: flex; justify-content: space-between;"> <div style="width: 20%;"> CAS No. <input style="width: 80%;" type="text" value="67-56-1"/> </div> <div style="width: 15%;"> Amount used: <input style="width: 20px;" type="text" value="1"/> <input style="width: 20px;" type="text" value="ml"/> </div> <div style="width: 15%;"> Period of use (hrs): <input style="width: 20px;" type="text" value="3"/> </div> <div style="width: 20%;"> The process is: <input style="width: 80%;" type="text" value="Semi Closed"/> </div> <div style="width: 20%;"> Physical State: <input style="width: 80%;" type="text" value="Non-Volatile Liquid"/> </div> </div>	<div style="border: 1px solid black; padding: 2px; display: inline-block; color: red; font-weight: bold;">OVERALL RISK:</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; color: green; font-weight: bold; font-size: 1.2em;">Medium</div>
W.E.L. (Itel / stel) <input style="width: 80%;" type="text"/>	<input type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested	<div style="border: 1px solid black; padding: 2px; display: inline-block;">Exposure Potential</div> <div style="border: 1px solid black; padding: 2px; display: inline-block; color: green; font-weight: bold;">Low</div>
Hazard Statement and Description	Precaution Statement and Description	+
H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhal	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H225 Highly flammable liquid and vapour.	P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.	X
H370 Causes damage to organs.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	X
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	X
	P240 Ground/bond container and receiving equipment.	X
	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	X
How will the precautions listed above be implemented?		
Refer to 'Precautions' section above for 'Fixation/Permeabilization Solution 1' - methanol is just a component of the solution		
Special Storage and Containment Measures	Disposal Method	+
Refer to storage considerations above for 'Fixation/Permeabilization Solution 1' - methanol is just a component of the solution	Refer to 'Disposal' section above for 'Fixation/Permeabilization Solution 1' - methanol is just a component of the solution	X
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.</i> Click here to see spill procedures	
Refer to 'Spillages' section above for 'Fixation/Permeabilization Solution 1' - methanol is just a component of the solution		

COSHH Form (Continued)

CHEMICAL NAME Fixation/Permeabilization Solution 2						Hazard Rating Low	OVERALL RISK: Low
CAS No. <input type="text"/>	Amount used 3 ml	Period of use (hrs) 3	The process is: Semi Closed	Physical State Non-Volatile Liquid	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential Low	
W.E.L. (Itel / stel) <input type="text"/>							

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?

There are no hazard/precaution statements for Fixation/Permeabilization solution 2. The solution does contain a small amount of Bovine Serum Albumin (BSA) which can be hazardous at high concentration (H302), however, the concentration of BSA in solution 2 is too small for it to be considered hazardous.

Special Storage and Containment Measures	Disposal Method	+
Container must be kept tightly shut in a fridge (2-8C). Container should not be kept in a freezer or exposed to sunlight. Formation of aerosols should be avoided,	While Fixation/Permeabilization Solution 2 is non-hazardous, it should not enter drains. Hence, any tissues/strippedetes/Eppendorf tubes with traces of Fixation/Permeabilization Solution 2 should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of Fixation/Permeabilization Solution 2 should be disposed in purple cytotoxic sharps containers.	x
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. Click here to see spill procedures</i>	

Any spillages are likely to be less than 1-3ml and within a BSC. For a small spill such as this, the spillage can be cleaned up using a tissue. 1:20 Chemgene will then be sprayed over the spill area which will also then be wiped down using a tissue. The tissues must then be disposed as cytotoxic solid waste.

CHEMICAL NAME 10x Permeabilization Buffer						Hazard Rating Low	OVERALL RISK: Low
CAS No. <input type="text"/>	Amount used 2 ml	Period of use (hrs) 3	The process is: Semi Closed	Physical State Non-Volatile Liquid	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential Low	
W.E.L. (Itel / stel) <input type="text"/>							

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?

There are no hazard/precaution statements for the 10x Permeabilization Buffer. The solution does contain small amounts of Bovine Serum Albumin (BSA) and Saponin which can be hazardous at high concentrations (H302 and H319 + H335 for BSA and Saponin respectively), however, the concentrations of both BSA and Saponin in the 10x Permeabilization Buffer are too small for it to be considered hazardous.



Special Storage and Containment Measures	Disposal Method	+
Container must be kept tightly shut at 21C Container should not be kept in a freezer or exposed to sunlight. Formation of aerosols should be avoided,	While buffer is non-hazardous, it should not enter drains. Hence, any tissues/strippedetes/Eppendorf tubes with traces of buffer should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of buffer should be disposed in purple cytotoxic sharps containers.	x
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material. Click here to see spill procedures</i>	

COSHH Form (Continued)

Any spillages are likely to be less than 1-3ml and within a BSC. For a small spill such as this, the spillage can be cleaned up using a tissue. 1:20 Chemgene will then be sprayed over the spill area which will also then be wiped down using a tissue. The tissues must then be disposed as cytotoxic solid waste.

CHEMICAL NAME						Hazard Rating High		OVERALL RISK: Medium					
Guava Instrument Cleaning Fluid (ICF)						Exposure Potential Low							
CAS No.	1310-58-3	Amount used	10 ml	Period of use (hrs)	2	The process is:	Open		Physical State	Non-Volatile Liquid	<input checked="" type="checkbox"/> Eyes	<input checked="" type="checkbox"/> Skin	<input type="checkbox"/> Inhaled
W.E.L. (Itel / stel)													

Hazard Statement and Description	Precaution Statement and Description	
H315 Causes skin irritation.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	+
H319 Causes serious eye irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
How will the precautions listed above be implemented?		
This is a chemical used for the Guava flow cytometer for which a COSHH has been completed and approved as part of the risk assessment for the 'Use and maintenance of Guava easyCyte 8HT benchtop flow cytometer' - refer to COSHH form in risk assessment SAF/MEME/940, 941		
Special Storage and Containment Measures	Disposal Method	
Refer to COSHH form in risk assessment SAF/MEME/940, 941	Refer to COSHH form in risk assessment SAF/MEME/940, 941	X
How will spillages be dealt with?		
Refer to COSHH form in risk assessment SAF/MEME/940, 941		

CHEMICAL NAME								Hazard Rating High		OVERALL RISK: Medium			
Sodium hypochlorite solution								Exposure Potential Low					
CAS No.	7681-52-9	Amount used	1.2 ml	Period of use (hrs)	2	The process is:	Open	Physical State	Non-Volatile Liquid		<input checked="" type="checkbox"/> Eyes	<input checked="" type="checkbox"/> Skin	<input type="checkbox"/> Inhaled
W.E.L. (Itel / stel)													

Hazard Statement and Description	Precaution Statement and Description	
H290 May be corrosive to metals.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	+
H314 Causes severe skin burns and eye damage.	P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.	X
H318 Causes serious eye damage.	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated	X
H400 Very toxic to aquatic life.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	X
H411 Toxic to aquatic life with long lasting effects.	P310 Immediately call a POISON CENTER or doctor/physician.	X
EUH031 Contact with acids liberates toxic gas.		X
How will the precautions listed above be implemented?		
This is a chemical used for the Guava flow cytometer for which a COSHH has been completed and approved as part of the risk assessment for the 'Use and maintenance of Guava easyCyte 8HT benchtop flow cytometer' - refer to COSHH form in risk assessment SAF/MEME/940, 941		
Special Storage and Containment Measures	Disposal Method	
		+

COSHH Form (Continued)

Refer to COSHH form in risk assessment SAF/MEME/940, 941	Refer to COSHH form in risk assessment SAF/MEME/940, 941	x
How will spillages be dealt with?	<i>Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.</i> Click here to see spill procedures	
Refer to COSHH form in risk assessment SAF/MEME/940, 941		

+ Add another chemical

Statement of work (Process to be undertaken)

Detection of treg cells following the co-culture of MSCs and CD4+ T cells or the culture of CD4+ T cells in MSC conditioned media.

Show Image

Personal protection requirements not covered in the precaution statements above.

Shoe covers

Sources of information and references

<https://www.miltenyibiotec.com/GB-en/products/treg-detection-kit-human.html#gref> - for treg detection kit data sheet; <https://www.stemcell.com/product-portfolios/t-cell-research/cell-activation-and-expansion/immunocult-human-cd3-cd28-t-cell-activator.html#section-product-documents> - for ImmunoCult™ Human CD3/CD28 T Cell Activator SDS and information sheet; SDS for Formaldehyde - see attached to email; <https://www.fishersci.co.uk/store/msds?partNumber=10010240&productDescription=5LT+Methanol%2C+Certified+AR+for+analysis&countryCode=GB&language=en>; SDS for Treg Detection Kit, human - see attached to email; <https://www.stemcell.com/products/human-recombinant-il-2.html#section-product-documents> - for Recombinant human IL-2 SDS and information sheet; SOP003; SOP039

Reference to **existing approved** Risk Assessment

SAF/MEME/940, 941; BRA

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/6749

Method Statement

SAF/MEME/6749

COSHH Assessment

SAF/MEME/982 - 989 & 9

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:

30 Mar 2022

Review comments