Safety Documentation

☑ **Method Statement** Reference SAF/MEME/7295

☑ **Risk Assessment** Reference SAF/MEME/7295

☑ **Chemicals COSHH** Reference SAF/MEME/1702 - 1707

School or Service	Wolfson School of Mechanical, Electrical and Manufacturing Engineering
Department	Centre for Biological Engineering
Originator name	Preeti Holland
email address	p.holland3@lboro.ac.uk
Location	CBE labs, Door H, Charnwood Building, Garendon Wing, Holywell Building
Project / Activity / Task	Scale up of Megakaryocyte cell culture
Supervisor Name	Professor Robert Thomas

Overall Assessment Scores

Risk Assessment	The activity is LOW RISK - and is effectively controlled
COSHH Risk Assessment	Low

Version : 2.33

Loughborough University
Centre for Biological Engineering

Safety Method Statement

Reference: SAF/MEME/7295

Location: CBE labs, Door H, Charnwood Building, Garendon Wing

Originator: Preeti Holland

Project / Activity / Task: Scale up of Megakaryocyte cell culture

What equipment will be used in this activity?

BD FACSCanto flow cytometer
Incubator
Class II Biological safety cabinet
Roller bottle platform
centrifuge
rocker platform
shaker platform
Cryostores
Autoclave
Light microscope
NucleoCounter-NC3000

What training must be completed to do this activity?

CBE Lab Induction
Biological safety cabinet training
Liquid nitrogen handling training
BD FACSCanto flow cytometer training

What chemicals are being used? (These must be included in the COSHH Form)

2-Mercaptoethanol (55mM)
Chemically defined lipid concentrate
0.5M EDTA solution pH 8.0
CryoStor cell cryopreservation media CS10
StemMACS™ CHIR99021 in DMSO
Dimethyl sulphoxide
CryoStor cell cryopreservation media CS10

Spill and accident procedures.

Safety Method Statement (Continued)

Unconfined spillages outside the biological safety cabinet. Small or low risk unconfined spillages (less that 10ml and generating little aerosol) may be cleaned up using the following procedures. Alert people in the immediate area of the spillage. Wash hands thoroughly and potentially other contaminated areas with soap and water. Wear clean disposable gloves and shoe covers. Use forceps or dustpan and scraper to remove sharps and place in sharps container. Use forceps or dustpan and brush to remove non-sharp solid material and place in an autoclave bag or container or yellow disposal bag as appropriate. Cover the spill area with paper towels soaked in 1% Virkon solution. Leave for 10 minutes then after this time place the soaked paper towels in a yellow biohazard bag for disposal. Wipe the adjacent area to the spill with 1% Virkon solution. Place all Virkon soaked paper towels, any other items with Virkon on them, and gloves into the yellow biohazard bag/container. All reusable PPE should be place in an autoclave bag for decontamination (as long as they don't have Virkon on them). Hands and other potentially contaminated areas should be washed again with soap and water. Inform lab staff and management when clean up is complete.
Large or higher risk unconfined spillages (more that 10ml or with considerable aerosol) are cleaned using the following procedures - 1) Alert other laboratory staff and try to avoid breathing the aerosol. 2) Leave the BSC on or switch it on and leave any cultures inside the BSC. 3) Leave the laboratory immediately or as quickly as possible. 4) Close laboratory doors and put up warning signs to prevent anyone from entering the lab. 5) Remove contaminated PPE or clothing and leave in the laboratory. 6) Wash hands or other areas potentially contaminated with soap and water. 7)Report incident to the laboratory manager and for significantly larger spills (>100ml) contact the local BGMSA and/or DSO for advice before proceeding. If authorized, proceed as follows; 8) wait at least 30 minutes for any aerosols to dissipate and assemble a clean up team of at least three people. 9) Put on appropriate PPE (lab coats, disposable gloves, shoe covers, safety goggles or full face shield and mask before entering the area of the spill. Determine the extent of the spillage and contain the spillage to avoid spreading. 10) Use forceps or a dustpan and scraper to remove any sharps or non sharp material and place into a sharps container/ autoclave bag/container or yellow disposal bag, depending on the nature of the spill and contaminants. 11) Cover the spill area with sufficient Virkon powder, from the outside of the spill area to the inside, to prevent aerosol generation. Leave for 30 minutes or until all liquid is absorbed. Scrape the soaked powder into a dustpan and place into a yellow biohazard bag/container. Wipe the adjacent area with paper towels and 1% Virkon solution. Place used paper towels into the yellow biohazard bag/container along with any disposable PPE. 12) Reusable PPE not contaminated with Virkon should be placed into an autoclave bag to be decontaminated. 13) Hands and other potentially contaminated areas should be washed again with soap and water. Inform lab staff and management when clean up is complete. 14) Seek medical assistance/treatment if required which is dependent on the nature and duration of exposure of the agent released.
For a confined spill within the BSC, alert people in the immediate area of the spill. Leave the BSC on for any aerosols generated. Put on clean disposable gloves and cover the spill area with paper towels soaked with 1% Virkon solution. Leave for 10 minutes. Use forceps or a dustpan and scraper to remove any sharps or non sharp material and place into a sharps container/ autoclave bag/container or yellow disposal bag, depending on the nature of the spill and contaminants. Wipe the adjacent area with paper towels and 1% Virkon solution. Place used paper towels into the yellow biohazard bag/container along with any disposable PPE. Disinfect gloved hands and remove gloves in the BSC. Remove any contaminated clothing and place in an autoclave bag for decontamination. Wash hands and arms thoroughly and put on a clean set of disposable gloves and protective clothing for the remainder of the clean up. Discard any bottles, flasks or solid material associated with the spill into the same container. Decontaminate any cultures, media or disposable materials adjacent to the spill area. Wipe the BSC walls, work surfaces, grills and the floor of the BSC and other items of equipment with 1% Virkon solution, then 1:20 Chemgene followed by 70% IMS. Items that are not easily surface decontaminated should be placed in autoclave bags for decontamination. All contaminated reusable PPE should be placed into an autoclave bag/container for decontamination (as long as they aren't overtly contaminated with Virkon) and all contaminated disposable PPE/items should be placed in a yellow biohazard bag. Hands and other potentially contaminated areas should be washed again with soap and water. Inform lab staff and management when clean up is complete. Allow the BSC to run for 10 minutes before resuming work.

Safety Method Statement (Continued)

For a confined spill within a centrifuge, close the lid and wait at least 30 minutes. Place a notice on the lid of the centrifuge to alert other lab users and inform the lab manager. If contamination is identified whilst centrifuge is running, turn off the centrifuge and do not open the lid for 30 minutes. Put on clean disposable gloves and full-face protection. After 30 minutes, open the lid and remove the buckets or rotor, wipe external surfaces with 1% Virkon and transfer to the BSC. Spray the interior of the centrifuge with 1% Virkon and wipe down the inside and all the parts of the lid with paper towels soaked in 1% Virkon. Rinse the surfaces with water or Neutracon (pH neutral), wipe down with 1:50 Chemgene followed by 70% IMS. In the BSC, open the buckets or rotors and retrieve any broken tubes with forceps and place in sharps container. Unbroken, capped tubes may be recovered and wiped with a 1% Virkon solution. Soak the buckets and lids in 1% Virkon for 10 minutes. Soak the rotor and attachments in 1% Virkon for 10 minutes then thoroughly rinse them with detergent or water and wipe with 1:50 Chemgene. All contaminated reusable PPE should be placed into an autoclave bag/container for decontamination (as long as they aren't overtly contaminated with Virkon) and all contaminated disposable PPE/items should be placed in a yellow biohazard bag. Hands and other potentially contaminated areas should be washed again with soap and water. Inform lab staff and management when clean up is complete. Inspect centrifuge before it is used again and do not use again until it has been authorised by the lab manager.
For a chemical spill, get away from the area and alert those in the immediate area. Identify the spill but do not go back to the area. Alert the laboratory manager and get help from them and others to assemble a team for clean up. Make sure the area has been sealed off and warn people of the hazard. If someone has been injured, get them to fresh air as soon as possible and seek medical help. Identify the hazard/chemical(s) involved, the physical state of the material, scale of the spillage, the location of the spill and people who could be affected by it. Wear the correct PPE to deal with the spillage and take the spillage kit to the site of the spill. Spread the absorbent material in the spill kit over the liquid spill. Sweep up the material using a dustpan and brush and place it in a disposal bag and label bag appropriately. Contact the DSO for waste disposal. If it is a solid chemical spill, use the plastic shovel to place spilled material into disposal bag. Once the bulk of the material has been cleaned up, wet a spill pad and wipe the area, and place the spill pad in the disposal bag. Wipe the area down with wet paper towels and dispose of then in the disposal bag along with the other waste from the spill clean up. Seal the bag with tape. Due to the hazardous properties of some chemicals or the size of the spill, assistance from the DSO may be required.
If a spillage contains both chemical and biological material, assess which type of material is most hazardous and proceed with the clean up for that material first following the above procedures.
Record all spills in the logbook or form FSOP038.1 and inform BGMSA/DSO of spillages and they will advice which forms to complete.
Report to University Health and Safety via the Online reporting system. Report any accidents where there may have been exposure to a pathogen or infectious material to Occupational Health and the University Health and Safety department.

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

Leave the BSC and incubators on and running
If it is safe to do so, turn off any electrical equipment
Make sure chemical bottles are closed or stored away safely in chemicals cabinets if possible
Leave the laboratory through the usual way
If the exit is blocked, remove the detachable window panel to the lab and use as an exit
If there is lab work ongoing, make sure any work is safe before exiting the lab to be left unattended. Use a ducted BSC for this work
If alarm sounds continuously evacuate lab in a safe and orderly fashion, evacuate building and head for appointed assembly point. Only return when informed that it is safe to do so

References.

Safety Method Statement (Continued)

SOP038 - Biological Spill Response
SOP039 - Storage, Handling and Disposal of Waste Chemicals and Solvents
SOP037 - Use of Personal Protective Equipment (PPE)
SOP003 - Decontamination & Disposal of Biological (Healthcare) Waste
SOP008 - Management and Control of Incoming Biological Material
SOP025 - Use and Maintenance of the Systec VX-95 Autoclaves
SOP031 - Cryopreservation and Storage of Mammalian cells
CBE BRA 204 - "Technology transfer and scale up of the differentiation of a human ESC line to megakaryocytes using the modified iMS10.8A cell line from University of Cambridge."
SOP013 - Safe Use and Maintenance of Liquid Nitrogen Stores
SOP032 - Revival of Cryopreserved Mammalian Cells

Detailed sequential description of the process

Process step	Precautionary measures and comments	
Receipt and cryopreservation of iMS10.8A cell line from University of Cambridge. Collaborators on project from University of Cambridge will send the cells once the Material Transfer Agreement (MTA) is in place. Cells will be received according to SOP008 and after inspection of packaging and vials, placed in a quarantine location in the cryostores. The material receipt checklist will be completed and all paperwork submitted to the lab manager.	The package received will be inspected for the integrity of the outer packaging and for any signs of leakage or other damage. Appropriate PPE (lab coat, gloves and safety glasses) will be work when opening the package that contains the vials of cells. Transfer of vials to liquid nitrogen cryostores will be carried out according to SOP013 and SOP031. An oxygen monitor will be used in the area where cryostores are used, doors will be propped open to ensure there is adequate ventilation, metal spill tray will be used, and appropriate PPE will be worn such as a face visor, covered footwear, lab coat and insulated gauntlet gloves.	
Culture and expansion of iMS10.8A hESC line to produce a cell bank of these cells for further experiments - Culture and expansion of adherent cells in Essential 8 growth medium over a few passages until there are enough cells to produce a cell bank (~ 25-50 million cells). Adherent cells will need to be dissociated from the tissue culture plastic using TrypLE and centrifuged, counted and seeded into further flasks for expansion. Cell culture media will be prepared following a protocol from collaborators at University of Cambridge and some supplements require a COSHH (see below).	On receipt of the cells from collaborators at University of Cambridge and approval of release, the cells will be cultured in tissue culture flasks and cultures will be expanded to have enough cells to generate a cell bank or stocks of cells for further experiments. All cell work will be carried out in a class II ducted BSC using aseptic techniques. Correct PPE will be worn (lab coat, nitrile gloves, shoe covers) and use of 1:50 Chemgene to wipe down any item being placed in the BSC and to spray gloves before handling culture flasks/plates. Centrifugation - carry out checks on centrifuge prior to use. Ensure correct rotor is fitted and correct settings for cells. Ensure tube and bucket lids are properly closed. Ensure centrifuge is correctly balanced before starting. Once started and up to full speed, check the centrifuge for any unusual noise or movement.	

Safety Method Statement (Continued)

Process step	Precautionary measures and comments
Cryopreservation of iMS10.8A hESC line and storage in liquid nitrogen cryostores - Following dissociation of adherent cell cultures from tissue culture flasks, cells are centrifuged, counted and the cell pellet is re-suspended at the correct cell density/ concentration in an appropriate cryopreservation medium such as CryoStor CS10 (see COSHH below) which contains 10% DMSO. The cell suspension is then pipetted into 1ml cryovials and the vials are placed into a "Mr Frosty" or Coolcell cryopreservation container. This is then kept at -80oc for at least 24 hours. The frozen cells are then transfered to cryostores for long term storage. Their location will be entered into Procuro and will be updated as necessary.	All cell work will be carried out in a class II ducted BSC using aseptic techniques. Correct PPE will be worn (lab coat, nitrile gloves, shoe covers) and use of 1:50 Chemgene to wipe down any item being placed in the BSC and to spray gloves before handling culture flasks/ plates. Handling of liquid nitrogen cryostores will be carried out in accordance with SOP031 and SOP013. An oxygen monitor will be used in the area where cryostores are used, doors will be propped open to ensure there is adequate ventilation, metal spill tray will be used, and appropriate PPE will be worn such as a face visor, covered footwear, lab coat and insulated gauntlet gloves.
Thawing of cells from liquid nitrogen cryostores - When iMS10.8A cells are required, vials will be retrieved from the cryostore and thawed for use in cell culture experiments. Vials are located using Procuro. The relavent cryostore is accessed and the correct rack is removed from the cryostore and placed onto a metal spill tray. The vial is quickly removed from the box and the box and metal support rod is put back in place on the cryostorage rack and returned to the cryostore and the lid placed back on a locked. Vials are thawed using a water bath at 37oc using a sample holder or foam float making sure the lid of the cryovial is not submerged. After a few minutes or until there is only a small amount of ice left in the vial, remove the vial from the water bath and wipe with IMS and place in a BSC. Dilute the thawed cell suspension in cryopreservation medium into fresh, warmed cell culture medium and centrifuge. Aspirate the supernatant from the cell pellet and then carefully tap the tube to break up the cell pellet. Resuspend the cell pellet in fresh cell culture medium and transfer to appropriate tissue culture flask/well plates.	All cell work will be carried out in a class II ducted BSC using aseptic techniques. Correct PPE will be worn (lab coat, nitrile gloves, shoe covers) and use of 1:50 Chemgene to wipe down any item being placed in the BSC and to spray gloves before handling culture flasks/ plates. Handling of liquid nitrogen cryostores will be carried out in accordance with SOP031 and SOP013. An oxygen monitor will be used in the area where cryostores are used, doors will be propped open to ensure there is adequate ventilation, metal spill tray will be used, and appropriate PPE will be worn such as a face visor, covered footwear, lab coat and insulated gauntlet gloves. Revival of cryopreserved cells will be carried out according to SOP032.
Differentiation of iMS10.8A hESC line into megakaryocytes - iMS10.8a cell line will be used in an inducible forward programming megakaryocyte differentiation protocol from University of Cambridge. The iMS10.8A cells have been genetically modified with an inducible cassette whereby differentiation is initiated from the inducible cassette with the addition of doxycycline to the culture.	A GMO biological risk assessment has been carried out for the iMS10.8A and has been approved (CBE BRA 204). Any chemicals for the differentiation medium that require a COSHH form have been added to this process risk assessment/COSHH form below. All cell work will be carried out in a class II ducted BSC using aseptic techniques. Correct PPE will be worn (lab coat, nitrile gloves, shoe covers) and use of 1:50 Chemgene to wipe down any item being placed in the BSC and to spray gloves before handling culture flasks/ plates.
Scale up of the differentiation of iMS10.8A hESC line into megakaryocytes using various platforms such as cell culture flasks, cell culture bags, roller bottles, and shaker flasks. Cultures will start at a smaller scale (tissue culture flasks/ small bottles/flasks <100ml total volume) and as volumes increase, the biological risk will be reviewed and any changes in necessary will be added to the risk assessment.	A GMO biological risk assessment has been carried out for the iMS10.8A and has been approved (CBE BRA 204). Any chemicals for the differentiation medium that require a COSHH form have been added to this process risk assessment/COSHH form below. The equipment used for scale up culture has been risk assessed below.

Loughborough University
Centre for Biological Engineering

Risk Assessment

Reference: SAF/MEME/7295

Location: CBE labs, Door H, Charnwood Building, Garendon Win

Originator: Preeti Holland

Project / Activity / Task: Scale up of Megakaryocyte cell culture

Category 1: Machinery & work equipment:			
Design and Construction	Mechanical hazards	Electrical hazards	Radiation hazards
N/A	N/A	Electrical test lables current	N/A

Category 2: Workplace
Falling/moving objects/materials
Risk of asphixiation (Oxygen depetion)
Localised cold surfaces
Slips/Trips/Falls on the level

Category 3: Hazardous and/or Harmful substances
Biological substancees (Infection)
Liquid Nitrogen / Cryogens
Chemicals used in process - COSHH below

Category 4: Work activity
Lone working out of hours

Category 5: Work organisation
N/A

Explain the risks associated with these hazards

People / Groups at risk: Operator only

Enter risk details here:-	Impact	Probability	Risk Score
Mis-use of cell culture platforms -roller/shaker/rocker	Slightly Harmful	Highly Unlikely	Low
What are the control measures?	**Lowers Impact**	**Lowers Probability**	
All laboratory users are trained to use the platforms and to ensure that culture vessels are placed on the platform correctly so that they do not fall off. The platforms are kept within an incubator to maintain the correct growth environment for cells.	Moderately	Moderately	

Process Risk Assessment Form (Continued)

			Residual Risk: Low
People / Groups at risk	Operator only		
Enter risk details here:-	Impact	Probability	Risk Score
Electrical hazard	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	
All electrical equipment is PAT tested every 2 years and all equipment is inspected prior to use to check cables and equipment for any damage, wear or looseness.	Moderately	Moderately	
User will ensure that cables are fed through the incubator door correctly and do not get caught in the either the incubator door or in the platform. Ensure that the incubator door is shut.	Moderately	Moderately	
			Residual Risk: Low
People / Groups at risk	Operator only		
Enter risk details here:-	Impact	Probability	Risk Score
Lone working	Slightly Harmful	Highly Unlikely	
What are the control measures?	Lowers Impact	Lowers Probability	
Operators are fully trained before being allowed to work out of hours	Moderately	Moderately	
Permission to work lone hours should be sought. All operators have a valid out of hours risk assessment for working out of hours detailing the work. They use the lone working app.using the following link. (https://www.lboro.ac.uk/media/wwwlboroacuk/content/healthandsafety/downloads/Lone%20Working%20App%20Instructions.pdf)	None	Moderately	
			Residual Risk: Low
People / Groups at risk	Operator only		
Enter risk details here:-	Impact	Probability	Risk Score
biological hazard - risk of infection	Harmful	Highly Unlikely	Low
What are the control measures?	Lowers Impact	Lowers Probability	
All biological material is contained and in lidded vessels.	Significantly	Significantly	
All operators are trained in cell culture and asceptic technique prior to starting work. All operators are trained to use cell culture platforms such as shaker, rocker and roller platforms.	Moderately	Moderately	
All biological material is risk assessed.	Moderately	Moderately	
			Residual Risk: Low
People / Groups at risk	Everyone in the room		

Process Risk Assessment Form (Continued)

Enter risk details here:-	Impact	Probability	Risk Score
Asphyxiation from liquid nitrogen	Harmful	Highly Unlikely	Low
What are the control measures?	**Lowers Impact**	**Lowers Probability**	
All operators are trained on liquid nitrogen handling and use of the crystores for long term cell cyropreservation. Only trained authorised users can access the cryostores.	Moderately	Moderately	
oxygen monitor present in the room	Significantly	Significantly	
Steady air change rate	Slightly	Moderately	
Assistance when using liquid nitrogen if necessary (two person team or informing someone else)	Slightly	Slightly	
		Residual Risk	Low

People / Groups at risk	Operator and people in proximity		
Enter risk details here:-	Impact	Probability	Risk Score
Slips trips and falls	Harmful	Unlikely	Medium
What are the control measures?	**Lowers Impact**	**Lowers Probability**	
Good housekeeping in lab Wear PPE specific to tasks Floor should be kept free of any trip hazards, especially true when handling nitrogen	Slightly	Moderately	
		Residual Risk	Low

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

Personnel Group	Maximum (Task setup/ Re-configuration)	High (Performing the task)	Medium (Observing the task)	Low (Present, but not involved)	Lone Working (Out of hours)	No Exposure Permitted	Total
Academic Staff	0	0	0	0	0	0	0
Technical Staff	0	1	0	0	0	0	1
Research Staff (PDRA)	0	1	0	0	0	0	1
Research Students (PhD)	0	0	0	0	0	0	0
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	2	0	0	0	0	2

With these controls in place, the risk is:

The activity is LOW RISK - and is effectively controlled

Loughborough University
Centre for Biological Engineering

COSHH Form

Reference: SAF/MEME/1702 - 1707

Location: CBE labs, Door H, Charnwood Building, Garendon Wing

Originator: Preeti Holland

Project / Activity / Task: Scale up of Megakaryocyte cell culture

CHEMICAL NAME

2-Mercaptoethanol

CAS No. 60-24-2

W.E.L. (Itel / stel) N/A

Hazard Rating: High

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State	Exposure Potential
0.5	ml	4	Semi Closed	Volatile Liquid	Low

Exposure Potential: ☐ Eyes ☑ Skin ☐ Inhaled ☐ Ingested

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description
H317 May cause an allergic skin reaction.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
	P280 Wear protective gloves/protective clothing/eye protection/face protection.
	P272 Contaminated work clothing should not be allowed out of the workplace.
	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
	P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
	P362 Take off contaminated clothing and wash before reuse.
	P501 Dispose of contents/container to approved waste disposal plant

Justify the use of this chemical:	This chemical is required for a specific cell culture protocol that we are working on in collaboration with University of Cambridge. This project is based on the technology transfer of this protocol from Cambridge to Loughborough and therefore the exact protocol has to be followed and the same chemicals must be used to carry out tech transfer.

How will the precautions listed above be implemented?

This chemical will only be used within a ducted biological safety cabinet as it is to be used for cell culture and it is a volatile chemical. All flasks and plates will be handled within a BSC. I will wear appropriate PPE such as a lab coat, gloves and safety goggles. The chemical will be used in small amounts as listed above. 2-Mercaptoethanol will be used in cell culture medium and therefore will be disposed of as biological waste containing cytotoxic/cytostatic substances. Waste will be disposed of using the purple/yellow stream. Solid waste will be disposed of in cytotoxic waste yellow/purple bags in yellow rigid containers. Contaminated pipette tips will be disposed of in purple-lidded yellow sharps containers.

Special Storage and Containment Measures	Disposal Method
This chemical should be stored in the fridge and as a safety precaution will be kept in secondary container with a fitted lid within the fridge with the hazard symbols displayed clearly.	Biological waste (See specific RA)

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

Absorbent cloth / tissue - if the spillage is chemical alone, use a chemical spill kit. If it is contaminated with biological waste, seek advice from the lab manager/DSO on how to proceed depending on whether it is the biological or chemical waste that most hazardous. Clean the most hazardous first and inform others in the laboratory about the spillage. See the spillages and accident procedures section of this form.

COSHH Form (Continued)

CHEMICAL NAME		Amount used		Period of use (hrs)	The process is:	Physical State	Hazard Rating	Exposure Potential	OVERALL RISK:
Ethyl alcohol (In Chemically Defined Lipid Concentrate)		5	ml	4	Semi Closed	Volatile Liquid	Medium	Low	Low
CAS No. 64-17-5									
W.E.L. (Itel / stel) 1000ppm									

Eyes
Skin
Inhaled
Ingested

Hazard Statement and Description	Precaution Statement and Description
H226 Flammable liquid and vapour.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
	P280 Wear protective gloves/protective clothing/eye protection/face protection.
	P233 Keep container tightly closed.
	P243 Take precautionary measures against static discharge.
	P240 Ground/bond container and receiving equipment.
	P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for ext
	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate
	P403 Store in a well-ventilated place.
	P235 Keep cool.
	P501 Dispose of contents/container to an approved waste disposal plant

How will the precautions listed above be implemented?

This chemical will only be used within a ducted biological safety cabinet as it is to be used for cell culture and it is a volatile chemical. All flasks and plates will be handled within a BSC. I will wear appropriate PPE such as a lab coat, gloves and safety glasses. The chemical will be used in small amounts as listed above. Eliminate all ignition sources, avoid breathing vapours or mists, use non-sparking tools and equipment, ensure adequate ventilation, use personal protection equipment. Chemically defined lipid concentrate (containing ethyl alcohol) will be used in cell culture medium and therefore will be disposed of as biological waste containing cytotoxic/cytostatic substances. Waste will be disposed of using the purple/yellow stream. Solid waste will be disposed of in cytotoxic waste yellow/purple bags in yellow rigid containers. Contaminated pipette tips will be disposed of in purple-lidded yellow sharps containers.

Special Storage and Containment Measures	Disposal Method
Keep in a dry, cool and well-ventilated place. Keep container tightly closed. Protect from light. Eliminate all ignition sources and keep in properly labelled containers. The reagent containing the chemical needs to be stored in the fridge. as a safety precaution it will be kept in secondary containment with fitted lid within the fridge with the hazard symbols displayed clearly.	Biological waste (See specific RA)

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

Absorbent cloth / tissue - if the spillage is the chemical alone, use a chemical spill kit. If the spillage contains biological waste, inform the lab manager/DSO and dependent of whether the chemical or the biological waste is most hazardous, clean that first and then the rest. Notify other lab users of the spillage. See the spillages and accident procedure section of this form.

COSHH Form (Continued)

CHEMICAL NAME

Glycine, N,N-1,2-ethanediylbis[N-

CAS No. 6381-92-6

W.E.L. (Itel / stel) N/A

Hazard Rating: High

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
1	ml	4	Semi Closed	Non-Volatile Liquid	☐ Eyes ☐ Skin ☐ Inhaled ☐ Ingested	Low

Hazard Statement and Description	Precaution Statement and Description
H373 May cause damage to organs through prolonged or repeated ex	P260 Do not breathe dust/fume/gas/mist/vapours/spray.
	P501 Dispose of contents/container to an approved waste disposal plant
	P314 Get medical advice/attention if you feel unwell.
	P501 Dispose of contents/container to an approved waste disposal plant

How will the precautions listed above be implemented?

This chemical is contained within a solution and this solution will only be used within a BSC. Appropriate PPE will be worn such as lab coat, gloves, shoe covers and safety glasses at all times in the lab. This chemical will be used in cell culture medium and therefore will be disposed of as biological waste containing cytotoxic/cytostatic substances. Waste will be disposed of using the purple/yellow stream. Solid waste will be disposed of in cytotoxic waste yellow/purple bags in yellow rigid containers. Contaminated pipette tips will be disposed of in purple-lidded yellow sharps containers.

Special Storage and Containment Measures	Disposal Method
Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers.	Biological waste (See specific RA)

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

Absorbent cloth / tissue - If the spillage is the chemical alone, use the chemical spill kit. If it is both the chemical and biological waste, inform the lab manager/DSO and depending on which type of waste is more hazardous, deal with that clean up first. Inform other lab users of the spillage. See the spillages and accident procedures section in this form.

CHEMICAL NAME

6-[2-[[4-(2,4-dichlorophenyl)-5-

CAS No. 252917-06-9

W.E.L. (Itel / stel) N/A

Hazard Rating: Medium

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.1	ml	4	Semi Closed	Volatile Liquid	☐ Eyes ☐ Skin ☐ Inhaled ☑ Ingested	Low

Hazard Statement and Description	Precaution Statement and Description
H302 Harmful if swallowed.	P264 Wash hands thoroughly after handling.
	P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unw
	P330 Rinse mouth.

How will the precautions listed above be implemented?

This chemical is contained within a solution and this solution will only be used within a ducted BSC. Appropriate PPE will be worn such as lab coat, gloves, shoe covers and safety glasses at all times in the lab. Avoid the formation of aerosol. Wash hands thoroughly after handling. This chemical will be used in cell culture medium and therefore will be disposed of as biological waste containing cytotoxic/cytostatic substances. Waste will be disposed of using the purple/yellow stream. Solid waste will be disposed of in cytotoxic waste yellow/purple bags in yellow rigid containers. Contaminated pipette tips will be disposed of in purple-lidded yellow sharps containers.

Special Storage and Containment Measures	Disposal Method
Keep container tightly closed. The following must be prevented: UV-radiation/sunlight. Storage temperature: -20°C. Avoid the formation of aerosol. Wash hands thoroughly after handling.	Biological waste (See specific RA)

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*

Absorbent cloth / tissue - if the spillage is the chemical alone, use the chemical spill kit. If the spillage is both chemical and biological inform the lab manager/DSO and seek advice if necessary. Inform other lab users of the spillage. Dependent on which is most hazardous, proceed with the clean up accordingly.

CHEMICAL NAME

Dimethyl Sulphoxide (DMSO)

CAS No. 67-68-5

W.E.L. (Itel / stel) N/A

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential	Hazard Rating
5	ml	0.5	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?

This chemical is not hazardous but lab coat, gloves and shoe covers will be worn at all times in the lab.

Special Storage and Containment Measures	Disposal Method
Store in a cool, well-ventilated area with the lid tightly closed. Combustible so must not be kept near sources of ignition.	If it is not diluted with FBS or used within a cryopreservation medium, it must be disposed of through the cytotoxic waste route. If it is diluted for use with cells, it can be disposed of as biological 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*

Absorbent cloth / tissue - if the spillage is the chemical alone use a chemical spill kit. If the spillage is both chemical and biological, inform the lab manager/DSO and seek advice on how to proceed. Depending on which is most hazardous proceed with clean up accordingly. Inform other lab users of spillage. See the spillages and accident procedures section of this form.

CHEMICAL NAME

Saccharose (In CryoStor ® cell cryopreservation media)

CAS No. 57-50-1

W.E.L. (Itel / stel) 20mg/m3

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential	Hazard Rating
50	ml	4	Semi Closed	Non-Volatile Liquid	Eyes, Skin, Inhaled, Ingested	Low	High

OVERALL RISK: **Low**

Hazard Statement and Description	Precaution Statement and Description
H290 May be corrosive to metals.	P234 Keep only in original container.
	P390 Absorb spillage to prevent material damage.

How will the precautions listed above be implemented?

This chemical is present within a solution that is going to be used for cell cryopreservation. The solution will be stored in the fridge and will only be kept in its original container. As a precaution the solution will be kept in secondary containment to ensure there are no leaks or spillages and the container will be clearly labeled. Appropriate PPE will be worn at all times such as lab coat, gloves, and shoe covers. This chemical will be used in cell culture medium and therefore will be disposed of as biological waste containing cytotoxic/cytostatic substances. Waste will be disposed of using the purple/yellow stream. Solid waste will be disposed of in cytotoxic waste yellow/purple bags in yellow rigid containers. Contaminated pipette tips will be disposed of in purple-lidded yellow sharps containers.

Special Storage and Containment Measures	Disposal Method
Store in the fridge and keep container tightly closed. Do not keep in a metal container. Combustible, corrosive material but requires storage at 2-8 degrees celcius.	Biological waste (See specific RA)
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*

Absorbent cloth / tissue - if the spillage is the chemical alone, use the chemical spill kit. If the spillage is contains both chemical and biological waste, inform the lab manager/DSO and seek advice on how to proceed. Depending on which is most hazardous, proceed with the clean up accordingly. Inform other lab users of the spillage. See the spillages and accidents procedures section of this form.

Statement of work (Process to be undertaken)

This process will be undertaken to carry out the technology transfer and scale up of the differentiation of the iMS10.8a human embryonic stem cell line (GMO HG1) to megakaryocytes. This work is being carried out in collaboration with the University of Cambridge and as such they have provided us with a protocol to follow and reproduce in the CBE labs. Once the technology transfer has been carried out successfully and the protocol has been reproduced, we will then start the scale up work to increase the yield of megakaryocytes generated using this protocol.

The iMS10.8a cell line has been received from University of Cambridge and a material transfer agreement is in place. The cell line has been genetically modified elsewhere before being received at the CBE. A biological risk assessment has been completed and approved for this cell line before the cells were received, as referenced below.

The iMS10.8a cell line will first be cultured and a master cell bank will be made so that there are adequate stocks of cells available for experiments. Briefly, cells will be thawed and cultured in tissue culture treated flasks then passaged and resuspended in cryopreservation medium. The cells will then be transfered into cryovials and stored at -80 degrees celcius for 24 hours. Following this, the vials will be transfered into liquid nitrogen for long term storage. Process steps are given in more detail above in this form.

For megakaryocyte differentiation, iMS10.8a cells will be thawed, cultured to confluency and then passaged. They will then be plated into well plates/flasks in differentiation medium following the protocol from University of Cambridge. This process starts off as adherent cell culture and becomes a suspension culture. At the suspension culture stage, we will investigate scale up of the process using various scale up platforms. Further information regarding this process has been detailed in CBE BRA 204 which has been approved before the work has started. The risk will be reviewed regularly as scale up begins and dependent on the volume of cell suspension being produced. The formation of megakaryocytes will be assessed using flow cytometry to identify key markers that are expressed both at the end point of the protocol and during the differentiation process.

The process steps are given in more detail in the process risk assessment part of the form.

Personal protection requirements not covered in the precaution statements above.

Shoe covers to be worn at all times in the lab. Cryogenic PPE (face visor, insulated gauntlet gloves, covered shoes) to be worn when handling liquid nitrogen.

COSHH Form (Continued)

Sources of information and references

SOP038 - Biological Spill Response
SOP039 - Storage, Handling and Disposal of Waste Chemicals and Solvents
SOP037 - Use of Personal Protective Equipment (PPE)
SOP008 - Management and Control of Incoming Biological Material
SOP003 - Decontamination & Disposal of Biological (Healthcare) Waste
SOP025 - Use and Maintenance of the Systec VX-95 Autoclaves
SOP031 - Cryopreservation and Storage of Mammalian cells
CBE BRA 204 - "Technology transfer and scale up of the differentiation of a human ESC line to megakaryocytes using the modified iMS10.8A cell line from University of Cambridge."
SOP013 - Safe Use and Maintenance of Liquid Nitrogen Stores
SOP032 - Revival of Cryopreserved Mammalian Cells
SOP004 - General laboratory housekeeping

Reference to **existing approved** Risk Assessment

CBE BRA 204 "Technology transfer and scale up of the differentiation of human ESC line to megakaryocytes"

With the current controls, the risk of using these chemicals is: Low

Supervisor to check that the process involving the safe use of these chemicals has been satisfactorily evaluated

Loughborough University

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: Rob Thomas — Digitally signed by Rob Thomas Date: 2022.08.12 10:40:54 +01'00'

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME/7295	SAF/MEME/7295	SAF/MEME/1702 - 1707

DSO Signature: Keven G Smith — Digitally signed by Keven G Smith Date: 2022.08.22 17:51:04 +01'00'

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: 22 Aug 2023

Review comments