

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	Angharad Evans
email address	a.evans@lboro.ac.uk
Location	Centre for Biological Engineering H29
Project / Activity / Task	Transient transfection of HEK293 / HEK293T with Polyethylenimine to produce viral vectors
Supervisor Name	Dr Elizabeth Ratcliffe

COSHH Form

Reference

Location

Originator

Project / Activity / Task

CHEMICAL NAME								 		Hazard Rating <input type="text" value="High"/>		<input type="text" value="X"/>	
<input type="text" value="Polyethylenimine, Branched"/>										Exposure Potential <input type="text" value="Low"/>		OVERALL RISK: <input type="text" value="Medium"/>	
CAS No. <input type="text" value="9002-98-6"/>		Amount used		Period of use (hrs)		The process is:		Physical State		<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested			
W.E.L. (Itel / stel) <input type="text"/>		<input type="text" value="0.132"/> ml		<input type="text" value="92"/>		<input type="text" value="Semi Closed"/>		<input type="text" value="Non-Volatile Liquid"/>					

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description	
<input type="text" value="H302 Harmful if swallowed."/>	<input type="text"/>	<input type="text" value="X"/>
<input type="text" value="H317 May cause an allergic skin reaction."/>	<input type="text" value="P280 Wear protective gloves/protective clothing/eye protection/face protection."/>	<input type="text" value="X"/>
<input type="text" value="H319 Causes serious eye irritation."/>	<input type="text" value="P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing."/>	<input type="text" value="X"/>
<input type="text" value="H411 Toxic to aquatic life with long lasting effects."/>	<input type="text" value="P273 Avoid release to the environment."/>	<input type="text" value="X"/>
Justify the use of this chemical: Chemical is a key component for transient transfection of cells and is one of the most widely used transfection methods amongst gene therapy manufacturers. The chemical will be used in small amounts 66ul per 6 well plate (11ul per well) in a BSC with all safety precautions adhered to.		
How will the precautions listed above be implemented? Following SOP037, all relevant PPE will be worn to ensure safe handling and avoid contact with skin. These include a standard side fastening white laboratory coat with elasticated sleeves, gloves, safety glasses and face mask. Gloves will be removed in accordance with good practice, without touching the outer surface, thereby avoiding skin contact with the substance. Once removed, used gloves will be disposed of as biohazardous waste (SOP003) and will be placed into the autoclave waste stream. The entire procedure will be undertaken within a BSC, thereby ensuring adequate ventilation and reducing the risk of inhaling any vapour or mist that might be produced. A face mask will also be worn as a precaution. If inhaled, the user will move into fresh air immediately. If a spill occurs, the substance will be soaked up with inert absorbent material and then disposed of as hazardous waste. It will be kept in suitable, closed, clearly labeled containers for disposal.		
Special Storage and Containment Measures	Disposal Method	
	<input type="text" value="Hydrophylic organic solvent waste"/>	<input type="text" value="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	
<input type="text" value="Absorbent cloth / tissue"/>		

+ Add another chemical

Statement of work (Process to be undertaken)

Transfection of Cells:
Cells are cultured as per manual cell culture until they reach a healthy 80% confluency before being split into either cell stacks, culture flasks or well plates. After seeding, cells would need to reach an approximate 60% confluency prior to Polyethylenimine (PEI transfection).



COSHH Form (Continued)

Prior to transfection, all reagents are heated to room temperature, and the BSC prepared as in SOP009 "Use and maintenance of HERASAFE KS Class II BSC". Once all reagents are RT a mixture containing PEI Pro and DMEM is made, and mixed by gently inverting the tube. A second mixture is made in a separate centrifuge tube, containing DMEM, a helper and packaging plasmid (pDG, Plasmid Factory, see GMO RA) and the lentiviral vector. This tube is also mixed then by inverting the tube.

The two mixtures are then combined and inverted once again before being incubated at RT for 15 mins. The reaction is then neutralised by adding the combined mixture to D10 medium (DMEM and 10% FBS) and mixed.

The final mixture is then split into the culture flasks / Cell stack / well plates that contain the cells and incubated for 72hrs at 37°C in 5% CO₂. Cells can be harvested 48hr, 72hr or 92hr post transfection.

Personal protection requirements not covered in the precaution statements above.

Sources of information and references

Supplier product: <https://www.sigmaaldrich.com/catalog/product/aldrich/408727?lang=en®ion=GB>

Supplier SDS: <https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=408727&brand=ALDRICH&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Faldrich%2F408727%3Flang%3Den>

Reference to **existing approved** Risk Assessment

CBE BRA 158 / CBEGMO 158

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

Method Statement

COSHH Assessment

CBE 293

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:

31/03/2020

Review comments