Document Number: **SASoM/METHOD/010.v6**

Title: **Thawing and Freezing of Cells for Cell Culture**

Version: **v6**

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SOP History		
Number	Date	Reason for Change
V1	01/01/2013	Original
v2	19/09/2013	Amendment applicable for SASoM
V3	19/09/2015	Biennial Update
V4	19/09/2017	Update
V5	19/09/2019	Update
V6	09/09/2021	Update

1.0 Purpose –

This SOP describes the current procedure for thawing and freezing down established cell lines in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved with thawing and freezing down of established cell lines.

3.0 Responsibilities –

All staff involved in the thawing and freezing down of established cell lines are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

All established cell lines held long-term within the SASoM are stored in Liquid Nitrogen located in the building. Access to the room is by means of a key along with your personal swipe card. Individuals using the liquid nitrogen facilities must have completed the appropriate training course before entering the liquid nitrogen facility.

Thawing Cells for Use in Cell Culture:

Identify the position of cells in Liquid Nitrogen tanks using the maps provided. Remove samples from the appropriate position and transfer samples to the research laboratory on dry ice. Subsequent work is carried out under sterile conditions in the cell culture suite.

Transfer approximately 20ml of appropriate cell culture media into a sterile 50ml centrifuge tube and label with information on (i) the full name of the cell line, (ii) the passage number, and (iii) the storage date as written on the vial. If the cells subsequently prove to be contaminated, this will allow us to identify other similar vials in storage which can potentially be seen as suspect and hence discarded.

Wipe the outside of the cryovial with 70% ETOH and let it air dry. Allow the sample to thaw out and then immediately transfer to the pre-labelled centrifuge tube containing media. Make up the volume to 25ml and then centrifuge at 1200rpm for 5min.

Discard the supernatant and add 1-2ml of fresh media to the cells. Break up the pellet with a pastette and then make up to a suitable volume with appropriate culture media. Transfer cell suspension to a suitable flask/plate and then place in the incubator.

Continue to culture cells in the usual manner.

Freezing Cells for Use in Cell Culture:

Trypsinise cells in the usual manner and then centrifuge at 1200rpm for 5mins.

Decant the media and initially resuspend the pellet in 1-2ml of Freeze Mixture (heat-inactivated foetal calf serum /10% DMSO). Break the pellet up with a sterile pastette and then make up to a suitable volume depending on the number of cells present. As a rough guideline, freeze the contents of a large 150 / 175cm flask into 5ml of Freeze Mixture.

Aliquot 5 x 1ml into sterile cryovials and label the vial with (i) the full name of the cell line, (ii) the passage number and (iii) the date these cells were frozen down on. Also write your own initials to identify who has frozen down the aliquot. Place cells in the -80ºc freezer in Mr Frosty pot prior to transferring into liquid nitrogen. Leave in the -80º freezer for no more than a week otherwise cell viability will be compromised.

Identify a suitable position for the cells in the Liquid Nitrogen tanks using the maps provided – see appropriate staff if you require additional storage space. Transfer

samples to the liquid nitrogen facility as soon as is practical and no longer than 1 week

Ensure that all maps are updated each time cells are added to / removed from the liquid nitrogen tank.

5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should under go training in this technique before performing the procedure.

All staff should complete an in house induction to the Liquid Nitrogen facility and the tissue culture area and be trained in sterile TC techniques before starting any TC work.

8.0 Related documents –

8.1 Risk assessments
RA20158_In Vitro Cell Proliferation Assays
RA22141_Preparation of Cell Media and additives

8.2 SOPs
SASoM/METHOD/008-Using Trypsin for Passaging Cells

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date: 09/09/2021
Management Approval:		
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date: 09/09/2021
QA release by:		
Name:	John O'Connor	
Position:	QA Manager	
Signature:		Date: 09/09/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE