Document Number:	**SASoM/METHOD/059.v4**
Title:	**Establishment of Three Dimensional (3D) Spheroids from Ultra Low Attachment (ULA) 96-well Trays.**
Version:	**v4**
Author:	**Peter Mullen**

Effective from:	10/11/2019
Valid to:	09/11/2021

SOP History		
Number	Date	Reason for Change
v1	18/12/2013	Original
V2	10/11/2015	Update
V3	10/11/2017	Update
V4	10/11/2019	Update

1.0 Purpose –
This SOP describes the current procedure for establishing three dimensional (3D) Cell Spheroids from Ultra Low Attachment 96-well Trays in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in cell culture work in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff involved in cell culture 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 –**

Three dimensional (3D) spheroids can be produced using either (i) Spinner flasks or (ii) Ultra Low Attachment (ULA) 96-well trays as per the appropriate SOP. This SOP details the procedure using Ultra Low Attachment (ULA) 96-well trays. Spheroids grown to date in this manner suggest that size and quality appears to depend on the cell line in question, some cell lines producing tight spheroids and others loosely-packed balls of cells.

Supplier of Ultra Low Attachment (ULA) 96-well Round Bottom Trays:

1. Culture of three dimensional (3D) spheroids using Ultra Low Attachment (ULA) Round Bottom 96-well trays can be problematic. It is therefore recommended that the following product is used for this protocol:

   Ultra Low Cluster, 96 Well, With Lid, Round Bottom, Ultra Low Attachment Corning #7007

Establishment of 3D Spheroids in Ultra Low Attachment (ULA) 96-well Round Bottom Trays:

1. Monolayer cultures are grown to approximately 80% confluence under normal growth conditions (eg DMEM supplemented with 10% heat-inactivated foetal calf serum (FCS) and penicillin / streptomycin (100IU/mL) in a humidified atmosphere of 5% CO_2 at 37°c).
2. Cells are then trypsinised in the usual manner to yield single cell suspensions.
3. Cells are counted using a haemocytometer and a cell count calculated.
4. Cell suspensions (1000 cells per well) are then transferred to each well in the 96-well tray. This may require further optimisation according to cell type.
5. The 96-well tray is then replaced in the humidified incubator (5% CO2 at 37ºc) and left for 48hrs prior to adding drug etc.
6. Media was changed weekly or as required and spheroid formation was assessed for up to three weeks. If spheroids have not formed after 48hrs it is unlikely that they will form thereafter.
7. Spheroids can be harvested and processed according to the end point of the assay.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

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 white tissue, then disinfected with 70% ethanol.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments
RA-BIOL-004 (Tissue culture)

8.2 SOPS
SASoM-METHOD-025 (Immunohistochemistry)
SASoM-METHOD-058 (Preparation of Spinner Flask Spheroids)
SASoM-METHOD-059 (Preparation of ULA Spheroids)
SASoM-METHOD-060 (Fixation of 3D Spheroids)

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
Management Approval:		
Name:	Peter Mullen	
Position:	SOP Administrator	
Signature:		Date:
QA release by:		
Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date:

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