Document Number:	**SASoM/METHOD/058.v4**
Title:	**Establishment of Three Dimensional (3D) Spheroids from Spinner Flasks**
Version:	**v4**
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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 Spinner Flasks 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) Low attachment 96-well trays as per the appropriate SOP. This SOP details the procedure using Spinner Flasks. A number of cell lines (of breast, ovarian, colorectal, and pancreatic origin) have been shown to produce spheroids in this manner although size and quality appear to depend on the cell line in question.

Establishment of 3D Spheroids in Spinner Flasks:

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 37ºc).
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 (1 - $5x10^6$ cells) are then transferred to sterile 'Spinner Flasks' and the centre cap (supporting the spinning bar) is made finger-tight. The two remaining smaller caps are similarly tightened but then loosened by ½ turn so as to allow gas exchange into the Spinner Flask.
5. The Spinner flask is then placed on a 'Cellspin' stirrer (Integra Biosciences) inside in a humidified incubator of 5% CO2 at 37ºc and the speed setting adjusted to '175' rpm. The incubator door is then closed with the apparatus inside.
6. Media was changed weekly or as required and spheroid formation was assessed for up to three weeks. If spheroids have not formed after 2-3 weeks it is unlikely that they will form thereafter.

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