Document Number:	**SASoM/METHOD/060.v4**
Title:	**Fixation of Three Dimensional (3D) Spheroids**
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 fixing three dimensional (3D) Cell Spheroids 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 (see section 8).

Fixation of 3D Spheroids from Spinner Flasks / 96-well trays for Histology:

1. Collect the spheroids by gently pouring the entire contents of the Spinner Flask into a 10-14cm Petri dish. Alternatively, hand-pick individual spheroids from the 96-well trays. *If you wish to continue culturing the remaining spheroids this must be done under aseptic conditions.*
2. Manually transfer selected spheroids to a small microcentrifuge tube with a sterile pastette and allow the spheroids to sediment down by gravity. Large spheroids will fall to the bottom like grains of sand!
3. Carefully remove the media, being careful not to disturb the spheroids at the bottom of the tube. Repeat steps 2-3 to add further spheroids to the tube if required and then again remove the excess media.
4. Add 100µL of Formalin.
5. Leave at room temperature for at least 30mins and no longer than a week prior to histology / immunohistochemistry.
6. Centrifuge the tube at the lowest speed possible (1000rpm for 2mins).
7. Remove the Formalin.
8. Add 50µL of molten agarose and gently 'stir' with a pipette tip to break up the pellet but hopefully conserve the integrity any spheroid formations. YOU MUST BE GENTLE at this stage of the procedure!
9. Allow the pellet to cool and process into paraffin blocks in the usual manner (this may be done externally at the Edinburgh University Division of Pathology, Western General Hospital, Edinburgh).,

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)
RA-COSHH-007 (Fixation and Staining of Cells)

8.2 SOPS:
SASoM-METHOD-025 (Immunohistochemistry)
SASoM-METHOD-058 (Preparation of Spinner Flask Spheroids)
SASoM-METHOD-059 (Preparation of ULA 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