Document Number:	**SASoM/METHOD/061.v5**
Title:	**Labelling of 3D Spheroids with 'Hypoxyprobe' reagent**
Version:	**V5**
Author:	**Peter Mullen**

Effective from:	01/02/2021
Valid to:	01/02/2023

SOP History		
Number	Date	Reason for Change
v1	18/12/2013	Original
V2	01/02/2015	Update
V3	01/02/2017	Update
V4	01/02/2019	Update
V5	01/02/2021	Update

1.0 Purpose –
This SOP describes the current procedure for Labelling of 3D Spheroids with 'Hypoxyprobe' reagent in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM involved in Labelling of 3D Spheroids with 'Hypoxyprobe' reagent 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 described in other Standard Operating Procedures (see section 8 below).

Labelling of 3D Spheroids with 'Hypoxyprobe' reagent:

Remove sufficient pre-formed spheroids from a spinner flask and transfer to a 60mm petri dish.

Experience suggests we should use 'Hypoxyprobe' at a final concentration of 100µM. Since 'Hypoxyprobe' is supplied at 20mM stock concentration, this will equate to a 1:200 dilution. 'Hypoxyprobe' is therefore made up at a working concentration of 5µL/mL (25µL/5mL media).

Remove all residual culture media from the petri dish and add 5mL of 'Hypoxyprobe' solution to each dish (final concentration 100uM). Return the petri dish containing spheroids to the incubator and leave for 45-60mins.

Remove spheroids from 'Hypoxyprobe' solution, transfer to 0.5mL microcentrifuge tubes, and then aspirate any residual media from the tubes.

Fix the spheroids in 4% Formaldehyde (50µL) before embedding in paraffin cutting sections as required.

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-004 (Formalin)

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:		
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Position:	Research Fellow	
Signature:		Date: 11/02/2021
Management Approval:		
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Position:	Research Fellow	
Signature:		Date: 11/02/2021
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Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date: 11/02/2021

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