Document Number:	**SASoM/METHOD/062.v4**
Title:	**Embedding of 3D spheroids in Agarose for subsequent IHC.**
Version:	**v4**
Author:	**Peter Mullen**

Effective from:	21/03/2020
Valid to:	20/03/2022

SOP History		
Number	Date	Reason for Change
v1	03/04/2014	Original
v2	21/03/2016	Update
v3	21/03/2018	Update
v4	21/03/2020	Biennial Update

1.0 Purpose –
This SOP describes the current procedure for embedding 3D spheroids in Agarose prior to IHC in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM embedding 3D spheroids in Agarose within Laboratory 248 at the St Andrews School of Medicine (SASoM).

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

4.0 Procedure –
Spheroids should be fixed in Formalin prior to embedding in agarose (see *SASoM-METHOD-060-Fixation of 3D Spheroids) for further details. As a general rule, samples should be in Formalin for 1hr per 1mm tissue thickness. Spheroids are usually transferred from the spinner flask to microcentrifuge tubes for fixing. And subsequent agarose embedding.*

Preparation of Agarose: Weigh out 2g of agarose powder (Fisher BPE-1356-500; melting point 88+/-1.5°C) and make up to 100mL with distilled water / PBS (final concentration is 2%). Melt in a microwave and dispense 5mL aliquots into sterile

universal containers. Store aliquots in the cold room. *N.B. Do not melt agarose on the FULL power setting.*

Procedure:

1. Carefully remove the formalin from the top of the spheroids using a fine-tipped pastette or a pipette.

2. Take one aliquot of agarose (5mL) from the cold room and melt in the microwave (approximately 20 seconds) until fully melted. Allow to cool at room temperature until it is hand hot, and then add 50 - 100µL of agarose to each sample tube. For larger spheroids add up to 500 µL per tube. Gently mix the agar and the spheroids using a long gel loading tip (these have a nice fine tip and minimize physical damage to the spheroids).

3. Allow the agar to set - this can be hastened by placing the container in cold water. Embedded spheroids can be left in the agarose plug at 4°C for up to a week prior to processing.

4. When set, the agar pellet can be removed from the universal container / microcentrifuge tube by inserting a small flat bladed spatula down the inner wall of the universal whilst rotating it simultaneously. This action will release the agar pellet which can then be trimmed down to a convenient size with the spheroids at the tip of the plug.

Samples can then be sent to a histology lab (Edinburgh University Dept of Pathology, Western General Hospital or NHS Lothian, NRIE) in order that they can be embedded in paraffin blocks. This is done by wrapping the cell pellet carefully in a small piece of speci-wrap (histological processing paper) and then placing into a labelled processing cassette before putting in the processor. The accompanying paperwork should indicate whether the specimen is for immunocytochemistry, for special stains or if specimen is very small.

5. Universals which have had formalin in them MUST NOT go in to autoclave bags. – discard small volumes down the sink with copious amounts of cold water.

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 paper towel, disinfected with 70% ethanol and finally washed with distilled water and a damp cloth.

7.0 Related documents –

7.1 Risk assessments – RA/COSHH/007 – Fixation and Staining of cells.

7.2 SOPs - SASoM-METHOD-060-Fixation of 3D Spheroids

8.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: Research Fellow

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