Document Number:	**SASoM/METHOD/134.v1**
Title:	**Preparation of Basal Culture Medium (BCM) for Organoid Cultures**
Version:	**v1**
Author:	**Peter Mullen**

Effective from:	09/02/2021
Valid to:	09/02/2023

SOP History		
Number	Date	Reason for Change
v1	09/02/2021	Original

1.0 Purpose –
This SOP describes the current procedure for making up Organoid Culture Media in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM making up Organoid Culture Media in Laboratory 248 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff making up Organoid Culture Media in this manner 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 –

'Organoid' cultures derived from primary tissue (e.g. colon) require a highly specific formulation of media which has been largely defined by the work of Hans Clever (Sato et al, 2011). Since the establishment of primary organoids from patient tissue is technically challenging, single cell suspensions of established cell lines were set up in Matrigel and cultured under fully supplemented Basal Culture Medium in order to (i) see if 'organoid-like' spheroids would develop using a 'hanging drop' technique , and (ii) see if such 'organoid-like' spheroids would continue to grow in the absence of Fetal Calf Serum (FCS).

This fully defined culture media does NOT contain fetal calf serum and is made up as shown in the table below:

Basal Culture Media Composition (for 3D Spheroids):

	500mL	**100mL**	**50mL**
DMEM / F12	500mL	100mL	50mL
Pen Strep (x100)	5mL	1mL	0.5mL
HEPES (x100)	5mL	1mL	0.5mL
Glutamax (X100)	5mL	1mL	0.5mL
B27 (x6=50)	10mL	2mL	1.0mL
N2 (x100)	5mL	1mL	0.5mL
hEGF	25.0ug	5.0ug	2.5ug
Nicotimnamide	610.6mg	122.12mg	6.106mg
N-acetyl-Cystein	815.95mg	163.19mg	81.595mg

Reagents:

Matrigel for Organoid Cultures: Corning #356255; Fisher #16491434 / A1413202; R&D Systems #3433-010-R1 (£509 / 10mL).
DMEM / F12 media; Fisher #A2494301 (£50.75 for 500mL).
Pen Strep ; Fisher #15140-122 (£15 per 100mL).
HEPES 100x; Fisher #15630-080 (£64.50).
Glutamax 100x; Fisher #35050-061 (£54.50).
B27 Supplement 50X; Fisher #17504044 (£79.50 for 10mL).
N2 Supplement 100X; Fisher 17502048 (£86).
Human rEGF; Fisher PHG0315 (£63.50 for 25ug).
Nicotinamide; Sigma N0636-100G (£30).
n-Acetyl Cystein; Sigma A9165-5G (£18.40)

Preparation:

DMEM / F12 Media: Fisher #A2494301 (£50.75 for 500mL).
Take out 1 x 500ml bottle of DMEM / F12 cell culture media from the cold room and place in a water bath at 37ºC to warm up. Supplements can be added to a smaller volume of media (e.g.50mL) in order to preserve stocks of (expensive) additives.

Pen Strep (x100) – Fisher #15140-122 (£15 per 100mL).

Store at -20°C. Thaw overnight at 4°C and then dispense into aliquots (20 x 5mL). Store the aliquots at -20°C before adding directly to media.

HEPES (x100): Fisher #15630-080 (£64.50 per 100mL).
Store at 4°C and then add directly to media.

Glutamax (x100): Fisher #35050-061 (£54.50).
Store at 4°C and then add directly to media.

B27 Supplement 50X; Fisher #17504044 (£79.50 for 10mL).
Thaw overnight at 4°C and then aliquot into working volumes of 1mL (10 x 1mL). Store at -20°C and do not freeze more than twice.

N2 Supplement 100X: Fisher #17502048 (£86).
Thaw in a water bath at 37°C until just thawed – avoid overheating. Use immediately or store in aliquots (10 x 0.5 mL) at -20°C.

Human EGF (25ug): Fisher #PHG0315 (£63.50 for 25ug)
Store the powder at 4°C. When ready to use, briefly centrifuge the vial and then reconstitute in 250uL of sterile PBS. Dispense into 10 x 25uL aliquots and freeze at -20°C. Each vial will contain a total of 2.5ug in a volume of 25uL.

Nicotinamide: Sigma N0636-100G (£30).
Weigh out the appropriate amount of Nicotinamide and then dissolve in a small volume of media taken from the media being made up. Filter sterilise before adding back to the media being made up.

n-Acetyl Cystein; Sigma A9165-5G.
Weigh out the appropriate amount of n-Acetyl Cystein and then dissolve in a small volume of media taken from the media being made up. Filter sterilise before adding back to the media being made up.

Matrigel:
Thaw on ice or overnight at 4°C – do not allow to thaw to room temperature! Aliquot the Matrigel (on ice) into sterile microcentrifuge tubes (0.5mL per tube) using chilled tips, tubes etc and then freeze the aliquots at -20°C. Each aliquot should be removed from the freezer immediately before use and not refrozen.

Procedure:
Cells were trypsinised, counted, resuspended in ice-cold Matrigel and then 25-50uL 'dropped' onto a pre-warmed 24-well tray (or other suitably sized petri dish etc). The tray (with its lid) was then quickly inverted (turned upside down) and placed in the incubator for 10 mins to polymerise. The tray was then turned the correct way up and the 'hanging drop' (which is now sitting on the bottom of the petri dish) is flooded with sufficient fully-supplemented culture media. Growth of the organoid was then monitored over a three-week period.

5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 detergent.

7.0 Training –
All staff should undergo training in this technique before performing the procedure.

8.1 **Related documents –**
8.1 Risk assessments
CHARM _RA22405_Preparation of Media for 3D Spheroid 'Organoids'

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

QA release by:

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