Document Number:	**SASoM/METHOD/020.v5**
Title:	**Preparation of Collagen Gels for Spheroid Invasion Assays**
Version:	**v5**
Author:	**Peter Mullen**

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Valid to:	13/03/2022

SOP History		
Number	Date	Reason for Change
V1	01/01/2013	Original
V2	14/03/2014	Additional information
V3	14/03/2016	Update
V4	14/03/2018	Update
V5	14/03/2020	Update

1.0 Purpose –
This SOP describes the current procedure for Preparing Collagen gels used for Invasion Assays in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved with preparing Collagen gels.

3.0 Responsibilities –
All staff involved in preparing collagen gels for Invasion assays 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 –

EVERYTHING WITH COLLAGEN MUST BE KEPT ON ICE at ALL TIMES!!!

Collagen Type 1 can be prepared in-house from rat tails (SASoM/METHOD/019; Preparation of Type 1 Collagen) or purchased commercially (Alpha Laboratories; #637-00653). From experience, collagen produced in house is highly variable (even within a single batch) and therefore it is recommended to purchase it commercially in order to get more reproducible results.

Amounts below will make 10mL of Collagen.

Preparation:

1. Prepare sterile distilled water (2 x 500mL) taken from the green Elga taps.
2. Place all tips, tubes, 24-well trays, sterile water, etc on ice to pre-cool.
3. Prepare Glacial Acetic Acid (1:1000) by diluting 200µL into 200mL of sterile distilled water. Place on ice to cool.
4. Prepare 0.22M NaOH by weighing out 2.2g of sodium hydroxide (powder or pellets) and making up to 250mL with distilled water. Filter sterilise using an Acrodisc 25mm Syringe filter (0.2µm Supor® membrane) manufactured by PALL (#PN4612) available from VWR (#514-4126). Place on ice to cool.

Making up the Collagen Solution:

1. Add 2.5ml of Collagen (Collage Type 1A; Alpha Laboratories; #637-00653) into a pre-cooled universal container.
2. Add 4.5mL of glacial acetic acid (1:1000).
3. Add 1mL of 10x Cell Culture Media.
4. Add 1mL of Heat-Inactivated Fetal Calf Serum.
5. Slowly add 1mL of 0.22M NaOH – the collagen/MEM mix should turn yellow to pale red colour as the pH is adjusted.
6. Gently swirl to mix and keep on ice until ready for use (0-30mins).

A: Pre-coating 96-well flat-bottom trays with Collagen.

1. Aliquot 50µL/well of the collagen mixture into the inner 60 wells of a 96-well tray. It is recommended that replicates of 6 are made for each set of conditions being studied.
2. Gently tap the side of the plate to ensure even distribution of the collagen in each well.
3. Incubate at room temperature for 2hr.
4. Aspirate residual unbound collagen from the plate.
5. Wash wells (x2) with sterile PBS or serum-free media.
6. Block with 1% BSA in PBS (0.25g / 25ml) by adding 100µL/well for 1hr at room temperature.
7. Aspirate residual BSA and proceed with assay or store according to ECM manufacturer's instructions.
8. Trypsinise cells of choice and then plate onto the collagen-coated wells.

B: Pre-coating 24-well flat-bottom trays with Collagen.

1. Dispense 500µL / well of the collagen mixture into each well of the 24-well tray. It is recommended that replicates of at least 4 be used if it all possible.
2. Gently tap the side of the plate to ensure even distribution of the liquid in each well.
3. Incubate at room temperature for 2hr.
4. Aspirate residual unbound collagen from the plate.
5. Wash wells (x2) with sterile PBS or serum-free media.
6. Block with 1% BSA in PBS (0.25g / 25ml) by adding 100µL/well for 1hr at room temperature.
7. Aspirate residual BSA and proceed with assay or store according to ECM manufacturer's instructions.
8. Trypsinise cells of choice and then plate onto the collagen-coated wells.

C: Setting up Collagen Gels with Spheroids derived from (i) Spinner Flasks.

1. Make up the Collagen mixture as detailed above – 10mL will be sufficient for approximately 15-18 wells. If spheroids are to be treated with drugs, the drug should be incorporated (at 1X concentration) into the collagen at this stage.
2. Aliquot the complete collagen mix into pre-cooled 24-well trays (0.5mL per well)
3. Decant spheroids from the spinner flask into a large sterile (10cm) petri dish.
4. Using a sterile pastette (cutting the tip off if necessary), transfer a single spheroid from the petri dish into each collagen well.
5. Transfer the 24-well tray to the incubator and leave for 1hr at 37°c for the collagen to set.
6. Add a further 0.5mL of culture media to each well and release the collagen plug from the well by running a pipette tip around the perimeter of the well. If spheroids are being treated with drugs, the drug should again be incorporated (at 1X concentration) into the media at this stage.
7. Replace the trays in the incubator for the remainder of the experiment.
8. Image the spheroids daily using conventional camera or Celigo scanner.

D: Setting up collagen gels with spheroids derived from (ii) Ultra Low-Attachment (ULA) 96-well trays:

1. Establish ULA spheroid cultures by placing 1000 cells / 200 µL (5000cells/mL) into each well of a 96-well round bottom ULA plate (Corning 7007; Scientific Laboratory Supplies).
2. Leave in the incubator for 4 days.
3. Carefully and slowly remove 100 µL of media from the well without disturbing the spheroid.
4. Place the 96-well tray on ice to cool (this can be done by placing two aluminium heat blocks side by side in an ice bucket and sitting the tray on top). Using pre-chilled tips, carefully and slowly add 100 µL of collagen solution (see above) to each well, again trying not to disturb the spheroid. If spheroids are to be treated with drugs, the drug should be incorporated (at 2X concentration) into the collagen at this stage.

5. Carefully transfer the 96-well tray to the cold room (where it should be level) and leave for 30mins to allow the spheroids to settle back down to the bottom of the well without the collagen setting.
6. Place the trays at room temperature for 1-2hrs to allow the collagen to set.
7. Top up the well with a further 100 µL of media (containing drug at 1X concentration) so that the final volume in each well should now be 300 µL.
8. Replace the trays in the incubator for the remainder of the experiment.
9. Image the spheroids daily using conventional camera or Celigo scanner.

5.0 Personal protection –
A Howie coat 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 detergent.

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

8.0 Related documents –

8.1 SOP SASoM/METHOD/008
Use of Trypsin for passaging cells in Cell Culture

8.2 SOP SASoM/METHOD/019
Preparation of Type 1 Collagen.

8.3 SOP SASoM/METHOD/021
Invasion Assays.

9.0 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